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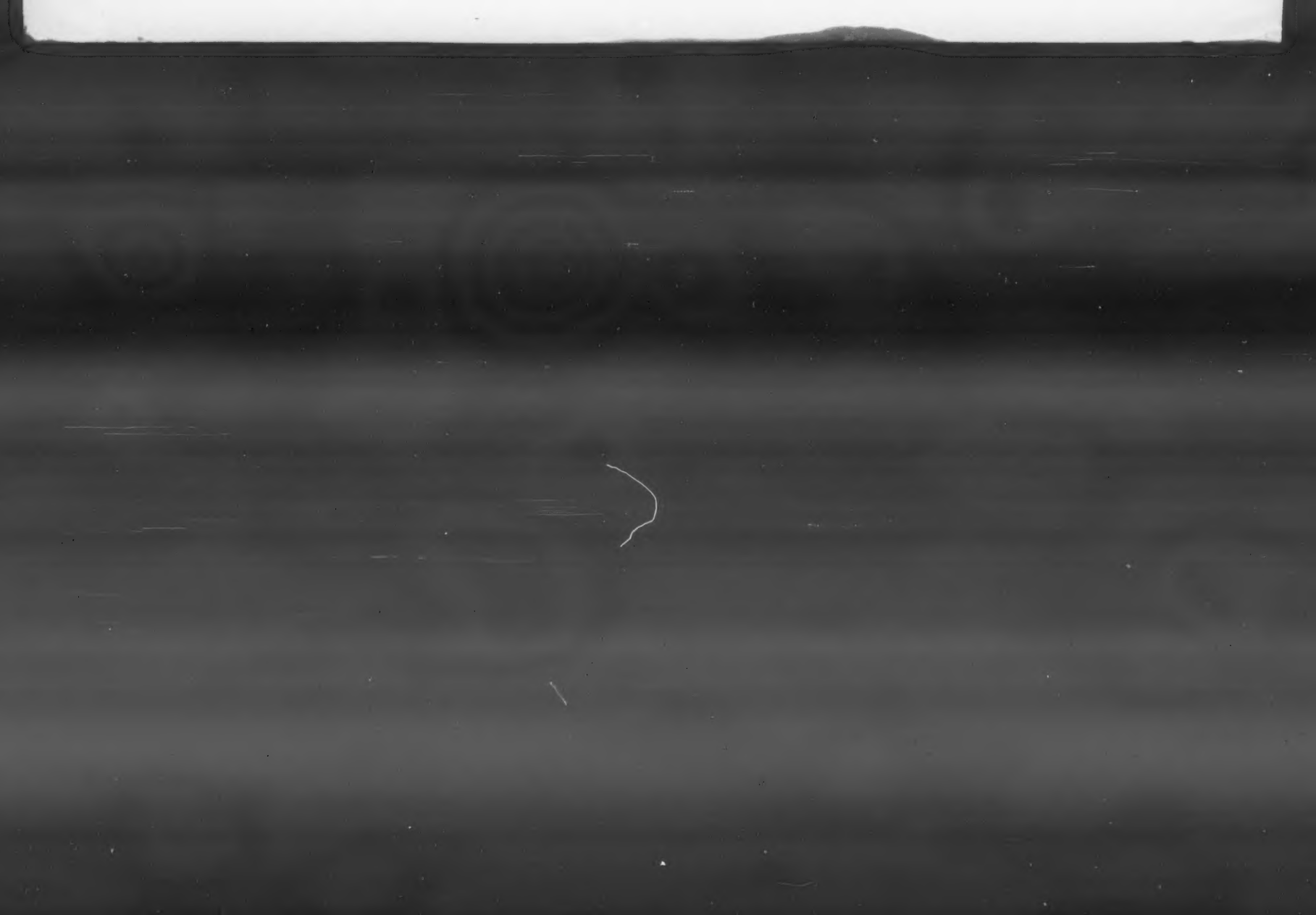

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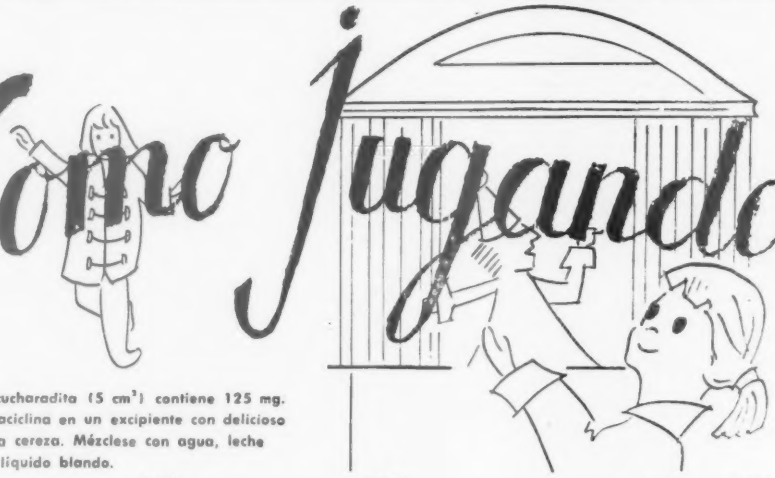

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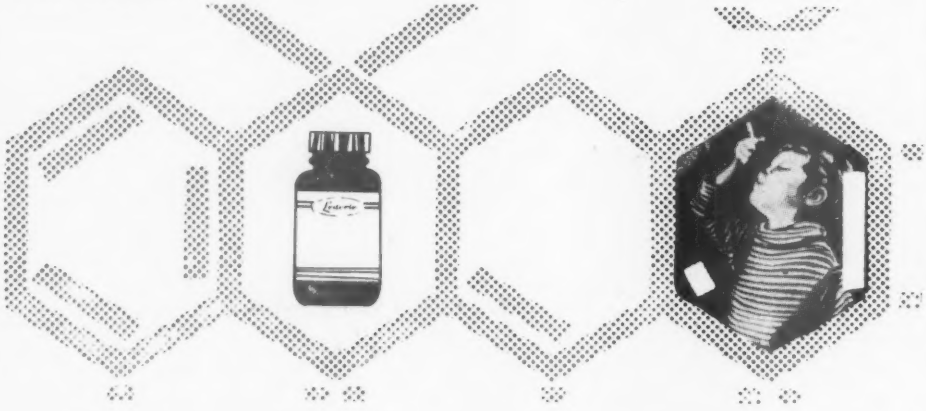

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ASOCIACION CIENCIA E INVESTIGACION
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ON THE DYNAMICS OF THE LUNG'S CAPILLARY CIRCULATION
III. THE EFFECTS OF ANOXIA

J. García Ramos and P. Rudomín *

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There are in the literature many papers establishing the importance of the oxygen tension in alveolar air for the control of pulmonary circulation. The early observation of von Euler and Liljestrand [1] of a rise in pulmonary arterial pressure in cats, after the animals were made to breathe 10 to 15 per cent oxygen in nitrogen, have been amply confirmed by many authors and even extended to man [2]. The currently accepted view is that during anoxia there is an increase in pulmonary vascular resistance. The presence of arteriolar vasoconstriction has been questioned, however, in view of the observation of Nisell [3] in which hypoxic blood perfused through cat lungs reduced instead the pulmonary vascular resistance. But this observation only led Nisell to shift the postulated constriction to the venules or the veins, an idea supported also by Peters and Roos [4].

As the rise in pulmonary arterial pressure has been observed in sympathectomized and vagotomized animals [1], as well as in isolated lungs [5], the vasoconstriction has been suggested to be produced either through axonic reflexes, or due to a direct action upon the blood vessels.

On the other hand, there is the posibility that the effect of anoxia does not depend from any direct vascular change. Drinker, based on his own observations [6], has suggested [7] that extravasation of fluid from capillaries with high permeability due to the anoxia would be the main reason for the increased vascular resistance. This idea has been questioned recently by Courtice and Korner [18].

There are, however, contradictory observations. For example, Reid *et al* [9] and Aviado *et al* [10] described pulmonary vasodilatation under anoxia. Duke and Killick [11] observed vasodilatation by cyanide or azide injected into the pulmonary artery. Leusen and Demeester [12] found an increase, as high as 96 per cent, in pulmonary vascular resistance in

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dogs which had received only the anesthetic. Dirken and Heemstra ([13]), in rabbits, observed the maximal effects only after 8 hours. Cournand ([14]) has attributed the increase in pulmonary arterial pressure observed in man, mainly to the increased cardiac output.

In view of the fact that we developed a method for detecting vasomotor changes in the pulmonary vascular bed ([15]), we decided to make some additional observations on this subjetc. We soon confirmed that there is an increase in pulmonary vascular resistance when the lung is ventilated with low oxygen gas mixtures. But we failed to correlate this increase with changes in the caliber of the lung capillaries or with significant vasomotor activity detected by oximetry. Since the first observations at the microscope, we found that there is a relatively rapid extravasation of fluid, and a filling of the alveolar sacs. For this reason, many of the experiments to be described were made with the idea of measuring this extravasation of fluid and correlate it with the observed changes in pulmonary resistance either at the vascular bed or at the airways (resistance to inflation).

METHOD

Several types of preparations were used. 1) Dogs anesthetized with pentobarbital (25-30 mg per kg), with either the chest closed or opened. 2) Rabbits under similar conditions, in which a lobe or a whole lung was ventilated through a different cannula. 3) Isolated perfused rabbit's lung, at room temperature (18°-20° C), and 4) the direct observation, throught the microscope, of the lung capillaries in *vivo* in the anesthetized rat, as described elsewhere ([16]).

The measurements included: arterial blood oxygen saturation by continuous oximetric recording; intrapulmonary pressure by a membrane manometer connected to the cannula while the lung or lobe was ventilated with a Starling type respiration pump (volume and rate being constants); systemic blood pressure with a mercury manometer; pulmonary artery pressure with a membrane or a mercury manometer, usually through a cannula placed in the main branch going to the inferior left lobe; total flow in the isolated and perfused lung; changes in weight in that same preparation; caliber changes of the capillaries in the rat lung, through photographs taken from the microscope field.

The gas mixtures employed were: atmospheric air; 50 per cent nitrogen in air; 75 per cent nitrogen in air; and pure nitrogen. For convenience they will be named later as air, 10 per cent oxygen, 5 per cent oxygen, and nitrogen, respectively. They were prepared in a respirometer (Collins type), from which the artificial respiration pump was fed. This procedure permited also the simultaneous measure of oxygen consumption under the different conditions explored.

In most of the lungs, specially in small animals, a thorough observation was made, at the end of the experiment, with the naked eye and at the dissecting microscope, of their outside aspect and in sections. In many of the isolated lungs the permeability of the vessels (artery to capillary) was judged at the end of the experiment by injecting india ink through the pulmonary artery, and watching the distribution of the coloured particles.

The measurement of the resistance of the lung to inflation was accomplished by recording intratracheal pressure while ventilating the lung with the Starling type pump. These results were more clearly apparent when the volume of air pumped was varied every few strokes, and several of those "steps" were thus recorded (figure 1). Usually, it was started with a large volume, then this was decreased step by step until a minimum was reached in which the pressure rise was almost negligible, and then, following backwards the same steps, the stroke volume was increased until the control value was reached again. In the intact animal the whole series was accomplished in about two minutes. This produces a certain degree of hypoxia to the lobe or the lung explored and the

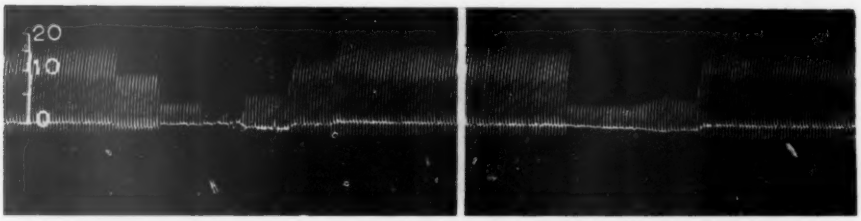

FIG. 1. — *Intratracheal pressure variations with reduction of the stroke volume of the artificial respiration pump. At the left, the reduction was made by steps. From 75 to 50, 25 and 12 ml, then the same values on the reverse order. Note that when the same steps are taken backwards the intratracheal pressure is correspondingly greater. At the right, the reduction was from 75 to 24 ml in one step. The increase in pressure gradually appeared and persisted for some time after coming back to the original 75 ml of stroke volume. Rabbit's lung* in situ. *Time marks, 20 seconds.*

condition was usually enough to cause changes in the lung tissue leading to an increase in its resistance to inflation, and also in the vascular resistance to flow.

In order to avoid complications for the measurements of intratracheal pressure, these records were taken with the thorax well open (usually in the midline, with the costal walls pulled at both sides and tied to the operating table). Sometimes the phrenic nerves were cut to paralyze the diaphragm. The only source of error remaining after this procedure, is the increase in venous return due to contraction of the abdominal muscles when some degree of general asphyxia was present.

RESULTS

1. *Relation between resistance to flow and resistance to ventilation.* This relation was first tested in the isolated perfused rabbit's lung. If the lung is perfused with saline (Ringer's) solution, edema progressively develops and there is a constant relation between the resistance both to flow and to ventilation. If the perfusion is made with blood or plasma,

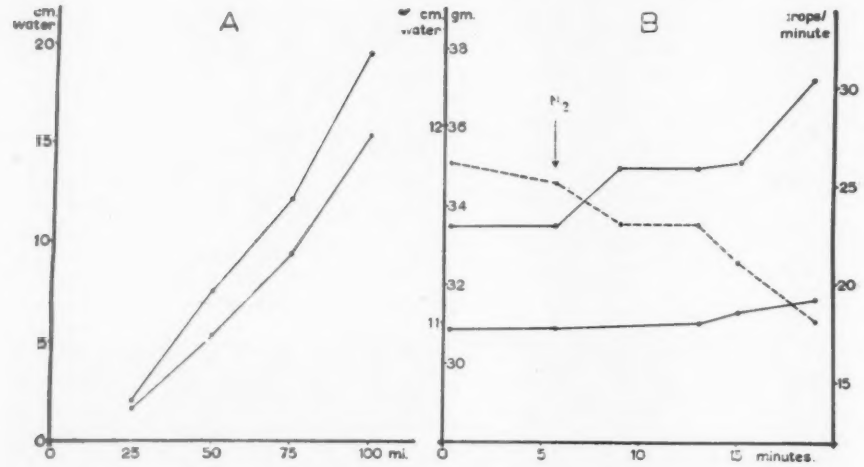

FIG. 2. — A. *Pressure-volume relations on a rabbit's lung ventilated with different gas mixtures and at several stroke volumes. The upper curve corresponds to ventilation with nitrogen, the lower one with atmospheric air.* B. *Temporal course of the variations in weight (continuous upper line), flow of perfusing fluid (interrupted line), and resistance to inflation (lower curve). Isolated rabbit's lung perfused with blood plasma. At the arrow, the ventilating gas was changed from air to nitrogen.*

the edema is established but at a very low rate. In this case, as in the previous one, the same relation holds. If the lung was ventilated with air as soon as the animal died from a blow in the back of the head, the preparation gave more clear differences, when it was ventilated with air and later with low oxygen gas mixtures (figure 2A). The resistance to inflation, as well as the resistance to flow, were greater when nitrogen was the ventilating gas (figure 2B).

The increase in resistance to inflation appears very clearly also in the lungs *in situ*, when, while ventilating with air, the stroke volume is reduced (figure 1). As this figure shows, if that reduction is made great enough in just one step, it takes only a few seconds for that resistance to be apparent, and it persists for some time after the basal conditions are regained.

The resistance to inflation goes up if the lung is ventilated with low oxygen gas mixtures. At this respect the rabbit's lung seemed to be more insensitive to anoxia than the dog's lung. For example, in a rabbit, after ventilating for 25 minutes both the middle and the lower lobes of the left lung with almost pure nitrogen, the increase in resistance to inflation was only 15 per cent. The rest of the lung was being ventilated with air. No signs of general anoxia were evident. At the end of that period, the nitrogen was substituted by air, the increase in resistance was not fully reversible, those parts of the lung put in anoxic conditions were showing clear signs of edema. On the contrary, in the dog of the

experiment from which figure 3 was taken, after about 5 minutes of ventilating the whole lung with pure oxygen, the mere change to air produced, after 5 minutes, an increase in resistance to inflation of about 6 per cent.

In the rabbit, after some time of ventilation with a very low oxygen gas mixture (5 per cent O_2, or less), the extra increase in resistance to inflation of a lobe or a whole lung, when the stroke volume of ventilating gas was reduced, did not appear or was very small.

In the intact animal, the resistance of the lung to inflation varies also in a parallel way to the vascular resistance, but only for mild conditions of hypoxia. It did not show, however, such a close relationship as in the isolated lung. In the dog, for example, when breathing 10 per cent oxygen was prolonged for several minutes, the resistance to inflation, after an initial period of gradual increase, began to fall with a similar course to the rise in systemic blood pressure and heart rate (figure 3).

In experiments of long duration, when the systemic blood pressure had reached low values, the resistance of the lung to inflation was lower than the control values and did not seem to vary much on ventilating the animal with the low oxygen gas mixtures. In these conditions, however, the pulmonary arterial pressure varied during anoxia in the usual way.

Some other conditions in which there was a change in the resistance of the lung to inflation were, for the case of the dog: a) after vagal stimulation, which produces a gradual increase in resistance up to 50 %, and b) following the occlusion of the inferior vena cava, in which the increase in resistance, to about 20 per cent, was abolished by bilateral section of the vagi.

It was further noted that following the conection of the artificial respiration pump with the respirometer there was a greater resistance of the lung to inflation which gradually subsided to an equilibrium. Later, when the respirometer was taken out from the circuit, the opposite change

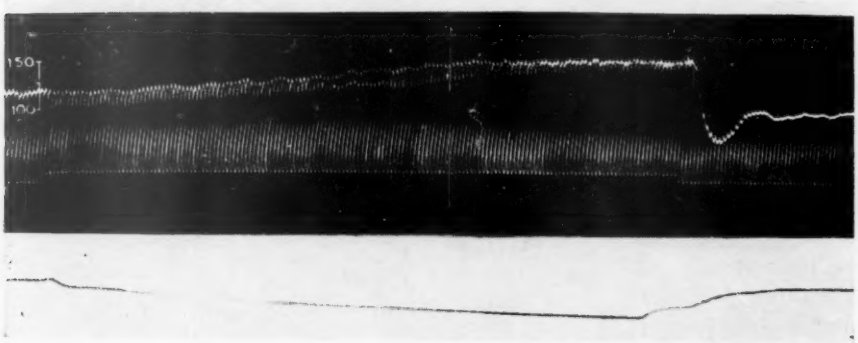

FIG. 3. — *Effects of breathing 10 % oxygen in an open-chest dog. From above downwards: systemic arterial blood pressure, intracheal pressure and arterial blood oxygen saturation.*

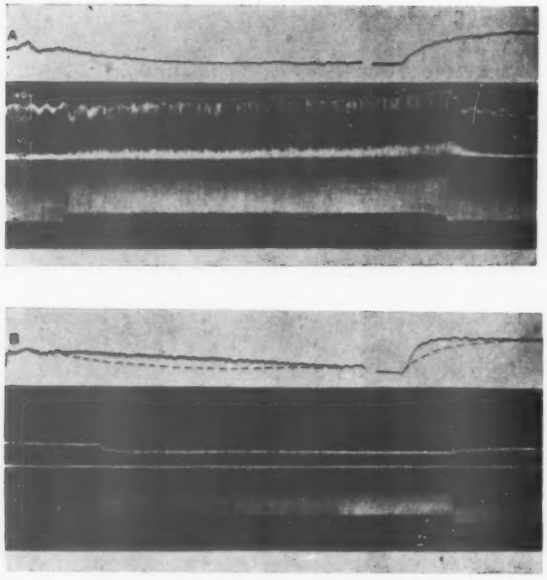

FIG. 4. — *Effects of 10 % oxygen, A before, and B after lung sympathectomy and removal of the adrenal glands were performed. Dog, chest opened. Tracings from above downwards: arterial blood oxygen saturation, systemic arterial pressure, pulmonary arterial pressure, and intratracheal pressure. The oxygram was taken at different speed from that of the kymogram, only initial and final portions are shown. In B the oxygram corresponding to A was superimposed (interrupted line, for better comparison).*

resulted, the resistance to inflation reached a transient minimum (figure 4). It must be noted that the respirometer in the breathing circuit introduces an additional resistance to the passive expiratory deflation of the lung.

2. *Changes accompanying the increase in resistance to flow and to inflation.* Parallel to the increase in resistance to flow and to inflation, there is an increase in weight, the course of which can be followed in the isolated perfused lung (figure 2B). Not only the weight but also the lung volume at expiration increases. In the rabbit, with the lung *in situ*, when the observation was prolonged, and the ventilation was made for long periods of time, or repeatedly, with the low oxygen gas mixtures, clear signs of pulmonary edema appeared. Not only at the microscope, but at the naked eye, the filling up of the air sacs with fluid, was a constant finding. At this point, india ink injected through the pulmonary artery distributed itself only in the regions of the lung in which the edema was less marked, usually the upper portions.

In the photographs taken from the same field, with about the same degree of lung inflation, in the rat, after ventilation with nitrogen, it was not possible to detect any change in the caliber of the vessels themselves.

3. *The relation between resistance to inflation, anoxia, and vasomotor changes in the intact animal.* In the second paper of this series ([15]) it was shown that a variation in arterial blood oxygen saturation, when ventilation is kept constant and velocity of blood flow is being controlled, can be taken to indicate a vasomotor change in the lung's "respiratory" vascular system. This same criterion was followed in the present study, although velocity of flow was not controlled. The observations made in

the anesthetized dog, with either opened or closed chest, showed a good correlation between resistance to inflation and the change in arterial blood oxygen saturation. The lung resistance increased as the oxygen saturation decreased, except for conditions of severe hypoxia mentioned above.

When the conditions of anoxia are not too severe, or when they occurred gradually, the oxygen saturation of arterial blood comes to an equilibrium with the corresponding oxygen partial pressure. There is no evidence of a significant vasomotor activity as judged by changes in arterial blood oxygen saturation. Before that equilibrium is reached, however, there are some oscillations in the saturation curves (figure 3). These oscillations did not appear after denervation of the lung.

If the oxygen content of the ventilating gas is suddenly changed, there are evident signs of vasomotor activity. As figure 3 shows, after some time of the start of a sudden decrease in the oxygen content of the breathing mixture, there is a vasoconstriction (a larger fall in saturation). This vasoconstriction in the pulmonary vascular bed follows a course similar to that of the rise in systemic arterial pressure. There is also a simultaneous and gradual rise in cardiac output as judged by the increase in pulse pressure in the pulmonary artery (figure 4A). All these changes were abolished after removal of both stellate ganglia and exclusion of the adrenal glands (figure 4B).

In the dog, even after ventilation with pure nitrogen for a period of about 5 minutes, or with low oxygen gas mixture for much more longer periods of time, there was no clear evidence of edema.

As an additional indicator of the conditions of the vascular bed of the lung during anoxia, the saturation curves of figure 4 can used. García Ramos ([17]) showed that, ventilation and cardiac output being constant, the rate of change in saturation must be an index of the functional surface area of the lung or, in other words, of the number of open capillaries present at that moment. A comparison of the two saturation curves in figure 4 shows that curve B has a higher rate of change that curve A, and this means that it should correspond to a condition in which the extent of the functional surface area of the lung is greater, therefore to a condition of vasodilatation, although the situation corresponding to curve *A* could well be due to vasoconstriction. Even if cardiac output was not controlled for this particular observation, a mere inspection to the records of systemic and pulmonary arterial pressure in each case indicates that the higher rate of curve *B* is due to vasodilatation.

Discussion

Before entering into discussion of the experimental results let us make an atempt to evaluate critically the methods employed. The lung resistance to inflation depends on several factors. a) A bronchomotor effect, which can probably be excluded in the isolated perfused preparation at room temperature. There is no reason to think that this phenomenon plays an important role, except perhaps for the decrease in resistance to inflation when the whole animal is under the effects of anoxia (figure 3). In this case, a bronchodilatation, due to the general sympatho-adrenal activity, seems a good explanation although it is not always

sufficient. Another two cases in which the state of the bronchial musculature might play a role are the effects of vagal stimulations and the changes due to occlusion of the inferior vena cava. b) The amount of fluid in the extravascular spaces. This is probably the most important factor for the lung of the rat and the rabbit. As described above, the correlation of that measure with the increase in weight and with the aspect of the tissue was always very striking. We can not say the same for the dog's lung. It was mentioned above that no signs of pulmonary edema were perceptible in any of the dogs used. Either the capillary permeability is not so altered under anoxic conditions, which is unlikely, or the lymphatic system is more efficient in this animal. c) The total amount of blood in the pulmonary vascular bed. This is probably the most important factor for the changes in resistance to inflation of the dog's lung, excepting those cases in which the bronchomotor factor had an important influence.

Summarizing, for the lungs of the rabbit and the rat, particulary in the isolated perfused preparations, the study of the resistance of the lung to inflation can be used as a criterion for changes in vascular resistance to flow. This would be due to the increase in the amount of fluid in the extravascular spaces. In the dog, since the interpretation is more difficult in view of the several factors involved, this measurement was used only as an additional evidence of the changes taking place in the lung.

In relation to the changes in arterial blood oxygen saturation, we think that they constitute a good method for detection of pulmonary vasomotor activity. The only possible source of error seems to be the piezometric effects on the alveolar capillaries brought in by the variations in cardiac output and the corresponding changes in velocity of blood flow ([16]). It was confirmed that this last mechanism plays a role, but its magnitude could be calculated and substracted from the actual results, leaving only that corresponding to the vasomotor activity. This is done by comparison of the results before and after denervation of the lung, together with removal of the adrenal glands. In this way, the method seems to be more sensitive that the simple measurement of pulmonary arterial pressure.

The observations presented here show that the increase in pulmonary vascular resistance due to anoxia is the result of an interplay of at least three mechanisms. First, there is an increase in permeability of the capillary membranes, with outflow of fluid and a corresponding loss of elasticy of the lung tissue. This loss of elasticity would affect both the vascular resistance to flow as well as the lung resistance to inflation (figure 2).

Secondly, there is some evidence of an active vasoconstriction of the arterioles during anoxia. This vasoconstriction would be due to a double mechanism. A reflex one forming part of the general sympathetic activity taking place under general anoxia, and another of humoral origin due to the adrenaline liberated by the adrenal glands. This last is the vasoconstriction abolished by removal of the stellate ganglia and exclusion of the adrenal glands (figure 4).

The direct effect of anoxia on the pulmonary blood vessels seems to be the same as the one seen on the vassels in other parts of the organism, namely vasodilatation. This would be the third mechanism. The increase

in resistance to inflation, shown in the first part of figure 3, can be interpreted in this way since a bronchoconstrictor effect can be reasonably excluded. The higher rate in the saturation curve of the figure 4B, points also in the same direction.

The proportion in which these three mechanism intervene in a particular case has to be variable depending on several conditions. In denervated preparations, as well as in the isolated perfused lung, the most important role seems to be played by the first one, the increase in permeability of the capillary membrane and the extravasation of fluid. Probably, this mechanism is also very important in the intact animal, at least this is true for the rabbit, but it can be reduced in importance by the efficiency of the lymphatics to remove the excess of extravased fluid. In man, it has been currently reported that a hypoventilated lung region gradually increase its opacity to X rays, and that the corresponding blood vessels do not fill with the opaque solutions used in clinical angiography. It is quite possible that the vasodilatation produced by anoxia might contribute to the extravasation of fluid and also to the X rays opacity, through the increase in total amount of blood contained in that territory. All those conditions would tend to exaggerate the state of poor ventilation through the increase in resistance to inflation.

An active vasoconstriction is probably of smaller significance in most cases. Of course it can be of considerable magnitude in animal experiments in which the conditions of general anoxia induce the activity of the sympatho-adrenal system. This does not mean to indicate that vasomotor activity is without importance in the normal animal. Vasoconstriction in the lung vessels affects very little the pulmonary arterial pressure, but the blood flow through the lung can be considerably altered. If not the total flow, the amount of blood going through the alveolar capillaries can be reduced. The vasomotor mechanisms of the lung would tend to maintain constant the perfusion of the alveoli ([15]).

In anoxia experiments, an important factor which might contribute to the rise in pulmonary arterial pressure is the increase in cardiac output due mainly to the sympatho-adrenal system activity.

SUMMARY

Some effects of ventilating a lung with low oxygen gas mixtures upon the pulmonary vascular beds were studied. It was confirmed that there is an increase in periferal resistance. About the mechanism of the increase the following observateions were made.

1. There is an extravasation of fluid from the capillaries very soon after ventilating the lung with low oxygen gas mixtures. The extravased fluid is clearly visible at the microscope in the rat's lung *in situ* as a filling up of the alveolar sacs, and as pulmonary edema in this an in the isolated perfused rabbit's lung preparation.

2. There is no visible change in the caliber of the lung capillaries, as judged through photographs taken from the microscope field in the rat's lung *in situ*.

3. There is an increase in lung resistance to inflation, both in the rabbit and the dog, with a parallel increase in the weight of the isolated and perfused preparation, and with the development of pulmonary edema.

4. If there is no evidence of general anoxia, there are no signs of vasomotor activity detected by changes in the arterial blood oxygen saturation with the lung's ventilation held constant and controlled cardiac output.

The experimental results described, seriously question the possibility of the increase in vascular resistance be due to vasoconstriction. On the contrary, give evidence that the direct effect of anoxia on the lung vessels is of vasodilatation. For example, during, or a short time after, breathing a low oxygen gas mixture, a saturation curve shows a faster rate of change than in the conditions under ventilation with air. This finding was taken to indicate that anoxia induces an increase in the functional surface area of the lung. In other words, that the number of active capillaries increase. With constant cardiac output this can be interpreted to be due to vasodilatation ([17]).

The conclusion is drawn that the increase in lungs' vascular resistance under anoxia is produced by the extravasation of fluid and subsequent loss of elasticity of the tissue, perhaps also to some degre of compression of the capillary vessels. There is an active vasoconstriction, buy only when anoxia affects the organism as a whole. This vascular constriction is mediated through the increased sympatho-adrenal activity, as it was supressed after denervation and exclusion of the adrenal glands.

Resumen

Se estudiaron algunos efectos sobre los vasos pulmonares, producidos por la ventilación de este órgano con mezclas pobres en oxígeno. Se confirmó la producción de aumento en la resistencia periférica con elevación de la presión en la arteria pulmonar. En cuanto a los mecanismos de este aumento, se hicieron las siguientes observaciones:

1. Muy pronto después de cambiar la ventilación, de aire a una mezcla pobre en oxígeno, se observó que hay salida de líquido de los vasos capilares hacia los alvéolos. La observación se hizo en el animal vivo (rata) con el pulmón *in situ*. Esta extravasación de líquido es también perceptible en la preparación de pulmón aislado y perfundido del conejo.

2. En fotografías tomadas al microscopio, del pulmón de la rata *in situ*, no se apreciaron cambios visibles en el calibre de los vasos pulmonares.

3. Se registró aumento en la resistencia del pulmón a su distensión, tanto en el conejo como en el perro, con aumento parelelo del peso del órgano en la preparación aislada y perfundida, y con indicios claros de aparición de edema pulmonar (figura 2).

4. En ausencia de signos generales de anoxia, no hubo actividad vasomotora en el pulmón que pudiera ser registrada por cambios en la saturación de oxígeno de la sangre arterial. Siempre que la ventilación pulmonar fuera mantenida constante y se controlara el gasto cardíaco.

Los resultados experimentales presentados ponen seriamente en duda la posibilidad de que el aumento de la resistencia vascular sea debida a vasoconstricción. Por el contrario, muestran que los efectos directos de la anoxia sobre los vasos pulmonares son de vasodilatación. Por ejemplo, la figura 4 muestra que poco después de ventilar un pulmón con una mezcla pobre en oxígeno, la curva de saturación obtenida indica una

mayor velocidad de cambio que la obtenida en condiciones de ventilación con aire. Esta observación se toma como prueba que la anoxia provoca un aumento del área funcional del pulmón o, en otras palabras, que hace aumentar el número de capilares activos por alvéolo. Si el gasto cardíaco no ha variado, la observación anterior puede muy bien ser atribuída a vasodilatación ([17]).

En conclusión, se establece que el aumento en la resistencia periférica de la red vascular del pulmón en condiciones de anoxia, es debida a la extravasación de líquido con pérdida subsecuente de la elasticidad del tejido, y tal vez también a cierto grado de compresión de los mismos capilares por el líquido extravasado. Hay también una vasoconstricción activa, pero solo cuando las condiciones de anoxia han afectado a todo el organismo. Esta constricción vascular es debida al aumento consiguiente de la actividad del sistema simpático suprarrenal, ya que es suprimida después de la denervación del pulmón y de la exclusión de las cápsulas suprarrenales.

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ADRENAL CHANGES FOLLOWING GONADECTOMY IN MICE OF DIFFERENT STRAINS

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MUCH experimental evidence supports the hypothesis of a relationship between the activity of the gonads and the adrenal cortex. The points on which this hypothesis was based are the following: a) the fact that both organs have a common embryological origin from the "genital ridge" in the mesoderm; b) the presence in some species of a clear sex dimorphism of the adrenal cortex; c) the changes observed in the adrenals related to the reproductive cycle; d) the effects of gonadectomy and/or the administration of sex hormones upon the adrenals, and e) the effects of adrenalectomy and/or the administration of cortical hormones on the sex organs and sexual function [see reviews by Allen (1) and Parkes (3)].

Morphological changes in the adrenals 20 days after bilateral ovariectomy in rats have been described by Pinto (4) who found a significant decrease in adrenal size due to atrophy and degeneration of the "zona reticularis". He also found that these changes could be prevented by repeated administration of estrogen. Furthermore, since estrogen treatment failed to prevent adrenal atrophy in hypophysectomized animals, he concluded that in this species the adrenotropic effect of estrogen might be mediated by a hypophyseal mechanism.

In the mouse perhaps the most clear demonstration of a relationship between gonads and adrenals has been given by the studies initiated by Wolley and collaborators (8) and followed by many others. In brief these investigations demonstrated that in most strains of mice regardless of sex, gonadectomy induces in 4 to 8 months the development of adrenal cortical tumors producing sex hormones. In most strains these tumors are of the benign adenomatous type, but at least in one stock (Ce) these are carcinomas which can be transplanted retaining their sexual function.

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Further studies showed that mice of either the A [Smith ([5]), Smith *et al* ([6])] or C57 Bl. strains [Woolley *et al* ([8])] were the only two stoks in which adrenal tumors did not develop. On the other hand, studies carried out in hybrids showed that the tendency to develop adrenal carcinoma is dominant over the tendency to develop adenoma and this in turn is dominant over the non-tumor tendency.

Since the post-castrational adrenal tumor in mice is a rather late manifestation of a process presumably starting shortly after gonadectomy, it was decided to investigate the early changes in the adrenals produced by the removal of the sex glands in mice of different strains. Specifically, studies were made to ascertain: a) whether or not the early effects of gonadectomy and/or the administration of sex hormones upon the adrenals are similar to those previously observed in rats, and b) if that were the case, whether or not there would be any difference in adrenal response depending upon the strain of mice used.

Material and methods

Adult mice of both sexes of the Z(C3H), BALB/C, Ce, NH, A and C57 Bl. (subline 1) inbred strains and AZ F1 and ZCe F1 hybrids were used. Two months after weaning mice of each sex were divided into two experimental groups. One group was submitted to bilateral gonadectomy and the other kept as intact controls. Gonadectomy in females was performed by the lumbar approach and in the males by extracting the testes through a small incision made over the skin of the scrotum. Hemostasis was obtained by twisting the testicular pedicles held with hemostatic forceps, thus avoiding the use of ligatures.

Intact controls as well as castrate mice were kept without further treatment for 30 days and then sacrificed, both adrenals dissected out and dropped in a bottle containing 10 % formaldehyde. After fixation for 24 hours each pair of glands were freed of the accompanying fat under a dissecting microscope, blotted on filter paper for 1 minute and immediately weighed on a torsion balance sensitive to 0.01 mg.

In some experiments a group of gonadectomized females received subcutaneously 1 μg of estradiol benzoate suspended in 0.1 cc of peanut oil per mouse, once every other day for a period of 30 days, starting the day after the operation. Their corresponding controls received only the same amount of peanut oil.

Results

Sexual differences in adrenal weight. Table I shows the results obtained when the weights of the adrenals are compared between male and female mice of different strains. It is evident that in all groups the adrenals from the females are significantly heavier those from the males, the differences being 53 % in the Ce's, 52 % in the AZ F1 hybrids, 43 % in the BALB/C, 41 % in the Z(C3H), 31 % in the C57 Bl's and 25 % in mice of the A strain. It is also clear that mice of the Ce stock have the largest adrenals of all strains investigated whereas those of the A stock have the smallest, both in males and females.

TABLE I

Sexual differences in adrenal weight in mice of various strains and hybrids

Strain	Sex	Number of mice	Adrenal weight mg/100 g B. Wt. (mean)	Difference bet. male & female (per cent)
BALB/C	Males	12	19.92 ± 0.4	43
	Females	16	28.56 ± 0.3	
Z(C3H)	Males	10	15.43 ± 0.6	41
	Females	25	22.70 ± 0.5	
C57 Bl. Subl. 1	Males	10	19.00 ± 0.6	31
	Females	10	24.90 ± 0.5	
Ce	Males	10	20.00 ± 0.3	53
	Females	10	30.54 ± 0.6	
A	Males	10	13.01 ± 0.7	25
	Females	14	16.23 ± 0.3	
AZ F1	Males	10	15.49 ± 0.7	52
	Females	10	23.60 ± 0.6	

By comparing the mean adrenal weight in AZ F1 hybrids with those in the A and Z(C3H) parental stocks it is evident that the size of the adrenals in the hybrid group resembles that in the Z(C3H) group, indicating that the "type Z(C3H) adrenal" is transmitted to the F1 progeny as a dominant factor over the "type A adrenal".

Effect of gonadectomy and estrogen administration in females. The results are listed in Table II. Mice of the BALB/C stock showed a significant decrease in adrenal size 30 days after bilateral ovariectomy from 28.56 ± 0.3 to 22.55 ± 0.5 mg per 100 g of body weight. However, the administration of estradiol in dose of 1 μg per mouse, once every other day, prevented although not completely the effect of castration (25.10 ± 0.4 mg). In the group of Z(C3H) mice gonadectomy did not induce signi-

TABLE II

Effect of gonadectomy and of estradiol benzoate administration upon the adrenal weight in female mice of various strains and hybrids*

Strain	Group	Number of mice	Adrenal weight mg/100 g B. Wt. (mean)
BALB/C	Intact controls	16	28.56 ± 0.3
	Gonadectomized	16	22.55 ± 0.5
	Gonad. & estradiol	12	24.02 ± 0.6
Z(C3H)	Intact controls	25	22.70 ± 0.5
	Gonadectomized	18	22.49 ± 0.4
	Gonad. & estradiol	19	24.02 ± 0.4
C57 Bl, Subl. 1	Intact controls	10	24.90 ± 0.5
	Gonadectomized	14	23.34 ± 0.6
	Gonad. & estradiol	14	24.50 ± 0.6
A	Intact controls	14	16.23 ± 0.3
	Gonadectomized	14	16.71 ± 0.5
	Gonad. & estradiol	10	16.80 ± 0.6
Ce	Intact controls	10	30.54 ± 0.6
	Gonadectomized	12	34.58 ± 0.5
	Gonad. & estradiol	7	29.75 ± 0.9
AZ Fl	Intact controls	10	23.60 ± 0.6
	Gonadectomized	10	23.44 ± 0.8
	Gonad. & estradiol	10	28.98 ± 0.5

* Estradiol was administered in dose of 1 μg per mouse every other day during one month.

ficant changes in adrenal weight (22.70 ± 0.5 in the controls and 22.49 ± 0.4 mg in ovariectomized). Also in this stock treatment with estrogen in the castrate had a stimulating effect upon the adrenals which showed an increase in weight above the values observed in the non-castrate controls (24.22 ± 0.6 mg).

Neither gonadectomy nor estrogen treatment induced significant changes in the adrenals of mice of the A and C57 Bl (subline 1) stocks. In the A group the values were 16.23 ± 0.3 mg in the controls, 16.71 ± 0.5

mg in the castrate, and 16.80 ± 0.6 mg in the castrate treated with estradiol. Similary, in the C57 Bl. group the values were 24.90 ± 0.5 mg, 23.34 ± 0.6 mg, and 24.50 ± 0.7 mg per 100 g body weight, respectively.

In regard to AZ Fl hybrids it is clear that the females of this cross (A ♀ x Z(C3H) ♂) responded to both gonadectomy and estradiol in a similar fashion as did mice of the Z(C3H) parental stock.

Finally female mice of the Ce strain responded to ovariectomy by an increase rather than a decrease in adrenal size (30 54 ± 0.6 mg in the controls and 34.58 ± 0.5 mg in gonadectomized). Also, estrogen administration produced a depressant rather than a stimulating effect upon the adrenals (29.75 ± 0.9 mg).

Effect of gonadectomy in males. The results of this experiment are shown in Table III. Mice of the BALB/C, Z(C3H), NH, AZ Fl, and ZCe Fl strains showed a significant increase in the size of the adrenals following gonadectomy. In contradistinction mice of either the A or C57 Bl. stock did not show any significant adrenal change.

Here again the adrenals of the AZ Fl hybrids responded to castration in a similar fashion as those of the Z(C3H) parental stock, whereas the adrenals of the ZCe Fl responded as those of the Ce parent.

Discussion

The results reported confirm in the mouse the existence of a difference in size of the adrenal glands related to sex, these being larger and heavier in females than in males. It is also found that there are important strain differences. According to Deanesly (1) the sex difference is primarily due to a larger development of the inner zone of the cortex which is not present in the mature male. Mice of the Ce stock had the largest adrenals in both males and females, whereas mice of the A strain had the smallest in both sexes. Furthermore, the percentage difference in adrenal weight between males and females in the A stock was found to be the least as compared with the other strains.

It is interesting to note that the weight of the adrenals in hybrid mice resulting from the cross between A and Z(C3H) resembles that in the latter stock, thus suggesting that in this particular cross the "Z adrenal pattern" is transmitted to the Fl progeny as a dominant characteristic. This is in agreement with the results reported by Smith (3) in similar hybrids in regard to the dominance of the tendency to develop post-castrational adrenal tumors (Z pattern) over the non-tumor tendency (A pattern).

As to the early effects of gonadectomy and estrogen treatment in females the results were different depending on the stock considered BALB/C mice showed a decrease in adrenal size following castration while estrogen administration prevented this effect. Mice of the Z(C3H) and AZ Fl hybrids failed to show adrenal weight changes after castration but these increase in size after estrogen treatment. Neither mice of the A strain nor those of the C57 Bl showed any significant change in adrenal weight after either gonadectomy or treatment with estradiol. This latter observation has particular interest in view of the fact that both kinds of mice are known to be non-susceptible to development of post-castrational adrenal tumors.

TABLE III

Effect of gonadectomy upon the adrenal weight in male mice of various strains and hybrids

Strain	Group	Number	Adrenal weight mg/100 g B. Wt. (mean)
BALB/C	Controls	12	19.92 ± 0.4
	Gonadect.	12	22.76 ± 0.8
Z(C3H)	Controls	10	15.43 ± 0.6
	Gonadect.	10	19.96 ± 0.5
NH	Controls	10	17.29 ± 0.7
	Gonadect.	10	20.80 ± 0.6
Ce	Controls	11	20.00 ± 0.3
	Gonadect.	12	23.60 ± 0.4
A	Controls	9	13.01 ± 0.7
	Gonadect.	9	13.88 ± 0.7
C57 Bl. Subl. 1	Controls	11	19.00 ± 0.6
	Gonadect.	11	18.45 ± 0.7
AZ F1	Controls	14	15.49 ± 0.7
	Gonadect.	15	18.60 ± 0.5
ZCe F1	Controls	10	18.30 ± 0.5
	Gonadect.	11	23.30 ± 0.6

Finally and contrary to expectation, mice of the Ce strain responded to both gonadectomy and estradiol in an opposite direction. In fact, castration produced an increase rather than a decrease in adrenal size, whereas

estrogen administration had a depressant rather than a stimulating action upon the adrenals.

In regard to the effects of gonadectomy in males it is clear that in all strains with the exception of strain A and C57 Bl. castration produced a significant increase in adrenal size.

The correct interpretation of these findings is not as yet clear to us. Since the effects of the sex hormones upon the adrenals in rats have been found to be mediated by some sort of hypophyseal mechanism [Pinto (4)] one may be inclined to think that the variability in adrenal response we have observed in different strains of mice could be the resultant of either quantitative or qualitative differences in hypophyseal function in each particular stock. On the other hand, it could also be postulated that being the hypophyseal mechanism involved, the same in all the strains, the differences observed in adrenal response might be related to either quantitative or qualitative differences in adrenal tissue sensitivity depending upon the strain considered.

SUMMARY

Using male and female mice of different stocks, the effects of gonadectomy and of the administration of sex hormones were studied. The results were as follows:

1. A sex difference in adrenal size, being larger in females than in males was confirmed in all the stocks of mice studied.

2. Gonadectomy in female mice was followed in 30 days by a decrease in adrenal size in mice of the BALB/C strain. No changes were observed in the adrenals of mice of the Z(C3H), A, C57 Bl., and AZ F1 hybrids whereas in mice of the Ce stock castration produced an increase rather than a decrease in adrenal size.

3. Estradiol administration in dose of 1 μg per mouse once every other day for 30 days given to gonadectomized mice produced an increase in size of the adrenals in animals of the BALB/C, Z(C3H), and AZ F1 hybrids. The same treatment did not induce changes in mice of the A and C57 Bl. whereas it had a depressant rather than a stimulating effect on the adrenals of Ce mice.

4. Gonadectomy in males was followed in 30 days by an increase in size of the adrenals in all strains of mice investigated with the exception of those of the A and C57 Bl. stocks.

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FURTHER STUDIES OF THE HAEMODYNAMIC ACTIONS OF THE ETHYL-METHYL-ISO-OCTENYL-AMINE (EMOA)

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THE RESULTS of studies designed to analyse the general pharmacologic actions of ethyl-methyl-iso-octenyl-amine have recently been published by Pardo et al., 1956. The drug has been shown to selectively block parasympathetic ganglia and to invert the blood pressure responses to tetraethylammonium. Since these effects imply that it is likely to become a useful tool for pharmacological dissection, a more complete analysis of its haemodynamic actions was undertaken.

METHODS

The experiments consisted of 12 cat heart-lung preparations, three isolated perfused rabbit hearts, and sixty blood pressure recordings in 3 dogs, 12 monkeys and 45 cats. In some of the latter, simultaneous electrocardiagrams were taken; in others, records were made of peripheral resistance distal to a constant flow Sigmamotor pump perfusing the femoral of other vascular beds. The animals were generally anesthetized by the intravenous injection of a mixture of hexobarbital sodium (35 mg/kg) and barbital sodium (150 mg/kg); occasionally thiopental sodium (15 mg/kg) was used instead of hexobarbital sodium. In most experiments boold pressure was registered from a cannula in the carotid artery and injections were made through the cannulated internal saphenous vein. In experiments designed to measure peripheral resistance, provision was made for direct injection into the artery. The interrelations between EMOA and each of the following drugs were studied: epinephrine, nor-epinephrine, Dibenzyline, Hydergine, tolazo-

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line, tetraethylammonium and hexametronium. All drugs were administered in the form of their soluble salts diluted in normal saline.

RESULTS

Effects on blood pressure. — The first intravenous injection of EMOA in the cat, the dog and the monkey, generally resulted in an initial fall in blood pressure followed by a secondary pressor response. The relative importance of each of the componenth seemed to depend in part on the level of pressure at the moment of injection and on the

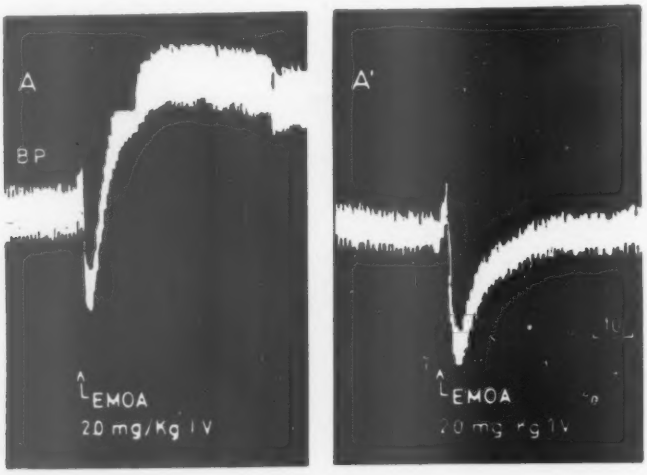

FIG. 1. — *Typical effects of EMOA on the blood pressure of the cat. Segment A presents the response to the first intravenous injection of 2.0 mg/kg of the drug; segment B, the response to the second equal dose administered 28 minutes after the first. Note the rapid tachyphylaxis to the pressor component.*

dose administered. With doses below 4.0 mg/kg the initial hypotension was of small magnitude. In the cat and in the dog tachyphyaxis to the pressor component developed rapidly at all dose levels, so that after the first injection subsequent doses produced only a fall in blood pressure, of magnitude and duration proportional to the dose administered (Fig. 1). In the monkey tachyphylaxis to the pressor component developed much more slowly and was sometimes still apparent after 15 doses of the drug administrated at 10 minutes intervals. Removal of the adrenals did not modify the blood pressure effects. In the open chest preparation, with vagi cut and stellate ganglia removed, the fall in pressure was more prominent but the initial pressor component did not disappear.

Effects on the heart. — The injection of EMOA in the anesthetized animal resulted in a lowering in heart rate which coincided with the fall in blood pressure. There was no secondary increase in heart rate, even after the first dose of the drug (Fig. 2). The electrocardiogram showed no alterations other than those attributable to changes in frequency. In the perfused isolated heart, relatively high doses (10 mcg/ml) produced a reversible negative inotropic effect, which coincided with a

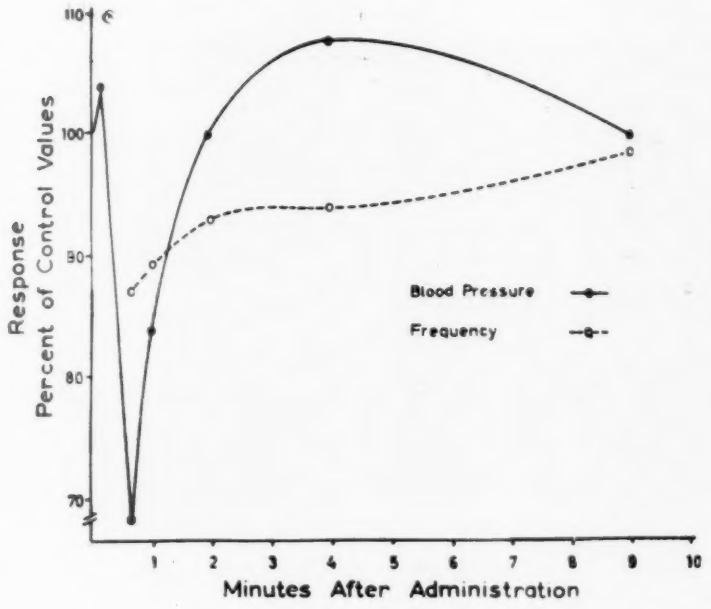

FIG. 2. — *Comparison between the effects of EMOA on the blood pressure and the heart rate. Responses to a first dose of 4.0 mg/kg of the drug in one of the experiments are transported graphically into terms of control blood pressure and frequency.*

moderate fall in frequency. During recovery, the frequency rose above the control level. In the heart lung preparation, low concentrations of EMOA increased, and high concentrations (greater than 20 mcg/ml) lowered the cardiac output, both effects out of proportion to the similar but smaller parallel changes in heart rate (Fig. 3). Both effects were reversible except with very high concentrations of the drug.

Effects on peripheral vascular beds. — When a large pressor component followed the first intravenous injection of EMOA, a parallel increase in peripheral resistance was observed in the femoral bed. When such initial pressor component was not apparent, and always, after tachyphylaxis had developed to it, the intravenous injection of the drug resulted in an initial transitory rise in peripheral resistance,

followed by a more lasting decrease. The intrafemoral injection was followed only by vasodilation, without the transitory increase in resistance. The intravenous injection of the drug ordinarily had no effect on the renal and celiac vascular beds. The intrarterial injection of large amounts into these areas was followedy by a decrease in peripheral

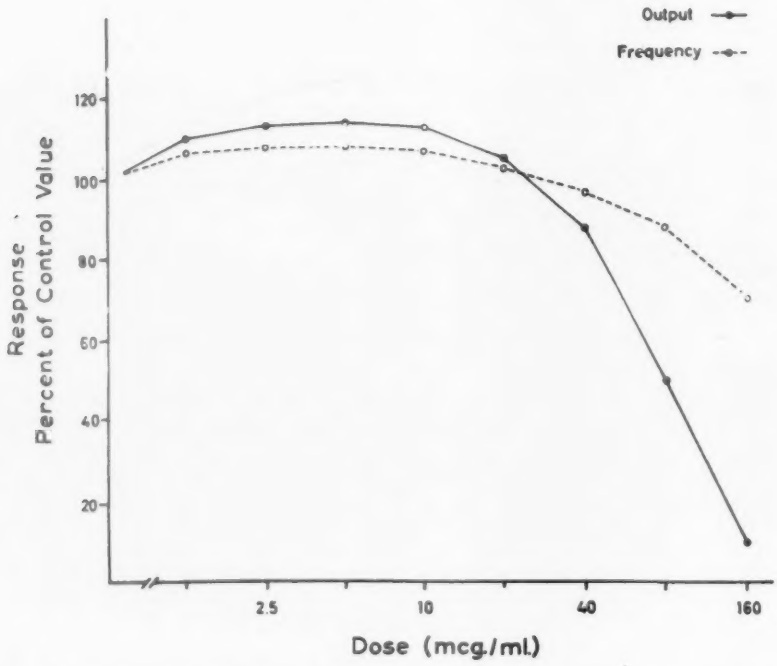

FIG. 3. — *Composite graph of the effects EMOA on output and rate in eight heart lung preparations. The abscissae represent the accumulated concentration of EMOA abtained by adding successive increments to the reservoir at approximately 5 minute intervals; the ordenate represent the mean maximum change for the elight experiments after each accumulated level of drug. After each eddition there was almost complete recovery to the orig.nal values, except with the highest levels of the drug. This recovery is not indicated in the graph.*

resistance. In the isolated perfused heart the addition of EMOA to the perfusion fluid disminished the coronary flow. (Fig. 4).

Interactions with other drugs.

Acetylcholine. — EMOA did not modify the cardiovascular effects of acetylcholine even in doses much larger than those required to block transmission of stimuli applied to the vagus.

Epinephrine and nor-ephinephrine. — An increase in the pressor response to nor-epinephrine after the injection of EMOA, similar to that already reported for epinephrine, was observed. The potentiation to both adrenergic mediators persisted after the blood pressure response to EMOA had passed. The reflex vasodilatation which follows the intravenous injection of epinephrine and nor-epinephrine was blocked by EMOA in doses that were without effect on the reflex response to the clamping of the carotids. During the continuos infusion of epi-

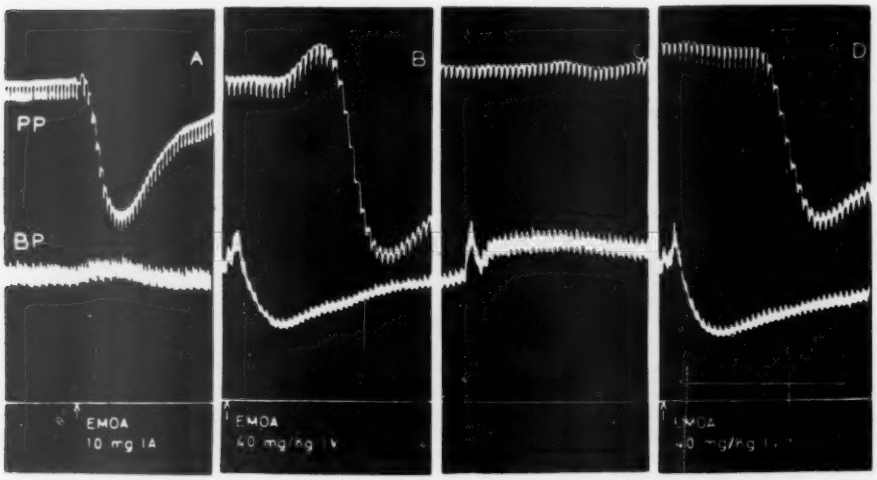

FIG. 4.— *Effects of EMOA on the blood pressure and the periperal resistance of the femoral bed in the cat, and on the changes in these parameters which follow the injection of hexamethonium. The upper record in each segment represents perfusion pressure measured distal to a constant volume pump perfusing one of the femoral arteries; the lower record represents blood pressure from the carotid. Control pressure was about 130 mm/Hg. The four segments are taken from the same experiment. A, shows the response to the intrafemoral injection 1.0 mg of EMOA; B, the response to the intravenous injection of 4.0 mg/kg of the drug (note the lag in response in the femoral bed and the initial reflex vasoconstriction, as also the lack of hypertensive component in the blood pressure recording); C, the response to the intravenous injection of 10.0 mg/kg of hexamethonium (note the absence of femoral response and the inversion of the typical blood pressure change); D, the response to a new intravenous dose of 4.0 mg/kg of EMOA (note the disappearance of the reflex constrictor component after hexamethonium).*

nephrine, the intravenous injection of EMOA resulted in a marked transitory fall in blood pressure.

Tetraethylammonium and hexamethonium. — After the administration of EMOA, the intravenous injection of TEA and C-6 produced a pressor response instead of the ordinary lowering of blood pressure

which follows such injection. The effect was of larger duration in the case of hexamethonium had no effect, or increased peripheral resistance in the femoral bed. The previous administration of TEA or C-6 did not alter blood pressure effects of EMOA. On the other hand, the injection of hexamethonium blacked the early reflex vasoconstriction which follows the intravenous injection of EMOA (Fig. 5).

Dibenzyline, Tholazoline and Hydergine. — After the administration of EMOA, the intravenous injection of the above adrenolytic agents resulted in a fall in blood pressure, though the effect is smaller than

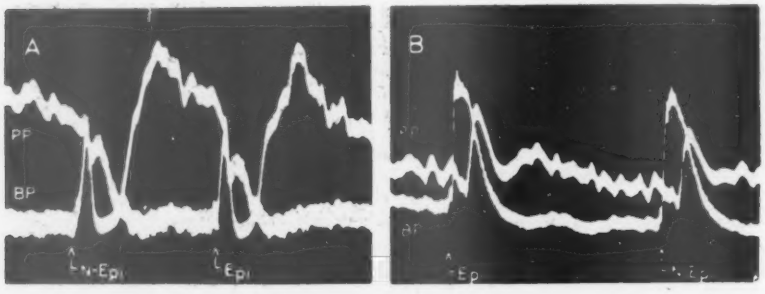

FIG. 5. — *Influence of EMOA on the effect of epinephrine and nor-epinephrine on the blood pressure and peripheral resistance in the dog. The upper record in each, segment represents perfusion pressure distal to a constant flow Sigmamotor pump perfusing one of the femoral arteries; the lower record represents blood pressure measured from the carotid. A, shows typical responses to each of the adrenergic mediators. B, chows the response to similar doses after the intravenous administration of 2.0 mg/kg of EMOA (note the disappearance of reflex vasodilatation).*

that observed in animals which had not received EMOA. The previous administration of these drugs did not appear to modify the blood pressure or the peripheral resistance responses to EMOA.

DISCUSSION

The present observations seem to indicate that EMOA has rather complex effects on the cardiovascular system other than that already reported of blocking transmission through the parasympathetic ganglia.

At the dose levels required to block ganglionic transmission, the drug produces a transitory lowering in cardiac output, lower doses may increase output. Very high doses result in a type of acute heart failure similar to that which results from the addition of barbiturates to the system. The possible effect of digitalis in this type of failure was not analyzed. The changes in frequency observed in the intact animal is probably a direct effect on the pacemaker. After the first dose, EMOA produces vasodilatation in the femoral bed and, with high intrarterial doses,

in other areas as well. The vasoconstriction which sometimes follows the first administration probably represents direct stimulation of effectors which are later blocked by the persistence of the stimulating agent. Tachyphylaxis to this effect is related to the similar poorly understood phenomenon observed in the case of sympathomimetic amines (Winder et al., 1948).

The observed data make it likelly that the fall in blood pressure which ordinarily follows the injection of EMOA is the combined result of the lowering in heart rate, a negative inotropic effect and a fall in peripheral resistance. The pressor component following the initial dose results from a direct action on the blood vessels.

The inversion of the blood pressure effects of TEA and hexamethonium suggests three possible interpretations. It coulr be that EMOA blocked transmission through the sympathetic ganglia, makink impossible a new decrease in adrenergic activity after the injection of EMOA or C-6 and unmasking possible direct actions of these drugs on the blood vessels or on the heart. Such a direct effect has been postulated for TEA (Moe and Freyburger, 1950). This, however, is not probable in the light of previous observations that indicate a lack of action on sympathetic ganglia, and in the light of the present observations that seem to indicate that some of the effects of EMOA itself (the reflex femoral vasoconstriction) require the integrity of the sympathetic efferent pathways. Moreover, two other considerations speak against this possibility: a) the fact that one dose of TEA does not invert the effect of the following dose and, b) the fact that EMOA does not block the blood pressure effects of adrenolytic agents. The second possibility is that EMOA could, in a manner similar to the constant infusion of epinephrine (Moe et al., 1949) supress "tonic" adrenergic vascular activity without involving the integrity of efferent sympathetic pathways also unmasking possible direct effects of TEA and C-6. The third possibility is that after EMOA, the ordinary blocking agents for such a possibility may be found in the effect of curare on a muscle which has been previously treated with 3-hydroxiphenyl-trimetylammonium (Riker and Wescoe, 1950). The matter deserves further experimental analysis.

The potentiation of epinephrine has been found to be a constant phenomenon, whatever the blood pressure effect of EMOA may have been, and to take place also in the case of arterenol. No new suggestion as to possible mechanisms involved is to be derived from the present observations.

It is interesting to consider that the blocking by EMOA of the reflex vasodilation which follows the intravenous injection of epinephrine and nor-epinephrine supports the contention (Gruhzit et al., 1954) that this effect may be mediated through the stimulation of vasodilator pathways. It would follow that in such pathways ganglionic transmis-

sion is more similar pharmacologically to that in parasympathetic ganglia and is therefore blocked by EMOA.

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ON THE VENTRICULAR STAIRCASE PHENOMENON

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ALTHOUGH the cardiac staircase phenomenon has been intensively studied ([1], [2], [3], [4], [5], [6]) its production mechanism is still unknown. Hoffman (1926) has reported that during its occurrence there exists a certain relationship between contraction and the action potential, which was recently confirmed in the isolated heart fiber by Trautwein and Dudel (1954) and by Niedergerke (1956). These findings have enhanced the significance of the process of myocardial activation in the mechanism of the staircase and hence deserve further elucidation.

Hajdu and Szent-Györgyi (1952), in addition to emphasizing the importance of ions in the onset of the phenomenon, have reported that blood serum appears to block its development in the frog heart. This fact suggests that in the intact animal the staircase would not be possible, an assumption we felt prompted to verify. Another issue at stake is the likelihood that the phenomenon be due to a progressive improvement in the oxigenation of cardiac fibers.

On the bases of the above considerations we have studied the correlation of electric and mechanical phenomena, as well as the influence of cooling and anoxia on the ventricular staircase. Moreover, we have thought that the mathematical study of the staircase curve might contribute useful data to further investigations or interpretations, wherefore such and analysis is included in this paper.

METHOD AND MATERIALS

Over 300 BUFO ARENARUM H. were employed. Experiments were conducted *in vitro* and *in situ*. *In vitro*, cardiac perfusion was made with Ringer-O^2 (Ringer: water 100, ClNa g 0.65, ClK 0.014, $CaCl^2$ 0.012, Co^3HNa 0.02, PO^4H^2Na 0.001), or with heparinized blood of BUFO. Sino-auricular or auriculo-ventricular ligature was made. The device employed for perfusion and recording has been described at length in a previous

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paper ([6]). Briefly speaking, the Poly-viso recorded both pressure and ventricular action potential, the latter gathered by 2 platinum electrodes applied over the external cardiac surface.

In the *in situ* heart, we made a sino-auricular ligature and recorded the tension set up by the ventricle at contraction (by attaching the apex to a strain gage and the latter to the Poly-viso), as well as the electric phenomena, as described earlier. The electric stimulation of the auricles or ventricles was conducted through the discharge of condensers or square pulses, the latter from a Grass stimulator, at a frequency ranging between 20 and 40 per minute, with rest intervals varying between 1-5 minutes in most experiments. For each stimulus, voltages between 5 and 20 volts and 1 milisecond duration, were used.

RESULTS

Normal staircase. THE BUFO ventricle, *in situ*, contracted in a staircase fashion, although the number of steps was nearly always smaller, given an identical rest period, than those of the ventricular staircase *in vitro*. The heparinized blood permitted gradual contraction. Other difference between toad blood and Ringer was that with blood inotropism proved greater. Neither the suppression of oxygen from the fluid during 40 minutes nor the anoxia resulting from the sole bubbling of nitrogen did suppress gradual contraction. In experiments using this gas it was found that after 1 hour bubbling in Ringer, contraction declined down to 40 % of its original value with O^2, probably owing to the hypodinamic state developed during anoxia. Thus, in one experiment with oxygen there were 7 initial steps following 1 minute rest and with nitrogen there were 9.

The most common feature of the (ascending) staircase of the BUFO in Ringer is that shown in figure 1 (typical staircase). Two unbound portions of the curve are observable: the initial, rapidly increasing one, followed by another portion growing slowly and persisting over a varying lapse, sometimes beyond 30 minutes. When, upon the completion of the staircase, frequency was reduced, the curve traced a descent of a roughly exponential pattern, depending upon the anterior and posterior frequencies. The absence of one single systole sufficed to cause the ventricle to re-start with a short staircase. This is indicative of the close dependence of the ventricle on rhythmic activation.

Following long intervals of cardiac rest, for instance, one hour, ventricular hypodynamia gave way to the development of a curve different from normal (atypical staircase, fig. 2); in it, the increase from one contraction to the next was always small. The possible significance of staircase curve pattern will be realized in the chapter concerned with mathematical study. The portion of small increase of the normal staircase was not always apparent; in fact, initial increase was followed by a slow decline of systolic tension, probably due to the myocardial stress caused by isometric activity.

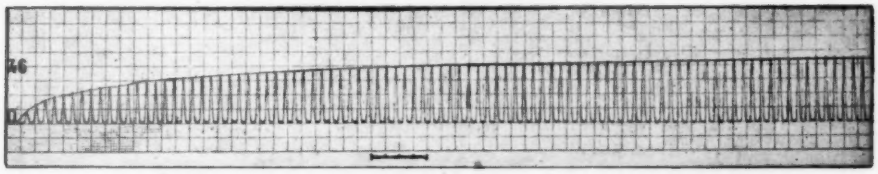
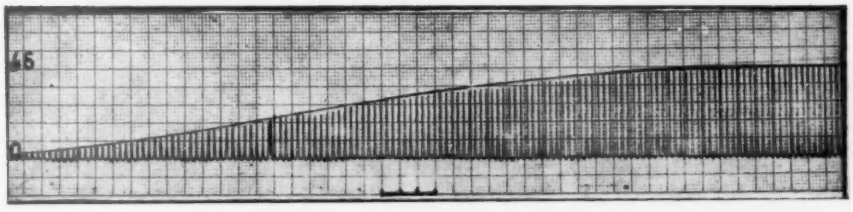


FIG. 1. — "Normal" staircase. *In abscissae: time in 5 sec. In ordinates: the ventricular pressure developed during contraction. The frequency of stimulation: 18 per minute, with one stimulus each 3.3 seconds. The quantitative values of the parameters defining* [15] *are:* $H_0 = 45.2$ *mm Hg;* $k_0 = 0.036\ s^{-1}$; $\lambda = 0.030\ s^{-1}$; $\mu = 0.82 \times 10^{-4}\ s^{-2}$. *The pressure values were corrected according to previous calibration prior to mathematical calculations. The comparison between the experimental record ant the theoretical curve adjusted shows very good agreement between data and hypothesis.*

Staircase and diastolic volume. Throughout normal staircase ventricular diastolic pressure underwent no changes. As established in an earlier paper (Mazzella, 1957) the progressive increase of diastolic volume affects the phenomenon mainly when ventricular overfilling takes place (fig. 3). Under such anomalous circumstances, the basal tension of fibers increases and the number of staircase steps diminishes. After the publication of the lastly named paper, the writers became acquainted with the study by Niedergerke on isolated heart fiber, his findings largely coinciding with the above mentioned ones. This latter author did not compare phenomena deriving from moderate or excessive distensions, for he makes no mention of staircase decline from over-tension. A conclusion is, however, inferable from the whole of the available data, namely that the staircase is not set up by the progressive increase in the diastolic length of the myocardium.

FIG. 2. — "Atypical" staircase. *In abscissae: time in 10 sec; in ordinates: ventricular pressure. The frequency of stimulation was also 18 per minute. The values of the parameters in the theoretical equation* [15] *are, in this case:* $H_0 = 46$ *mm Hg;* $k_0 = 0.005\ s^{-1}$; $\lambda = 0.035\ s^{-1}$; $\mu = 0.38 \times 10^{-4}\ s^{-2}$. *A previous correction of the ordinates recorded has also been made. Note that the rate of growth of this staircase is considerably slower than that in figure 1, because the first explosive factor has an initial value of approximately one seventh of the same value in the first staircase. This staircase reaches 99 % of its asymptotic value in 7 m 30 s while that in figure 1, in only 4 m 30 s. Nevertheless, the asymptotic value is practically equal in both curves.*



Cooling. Anoxia. Hajdu and Szent-Györgyi have found that the staircase is reduced as a result of cooling. Progressive or abrupt cooling, involving a temperature drop of Ringer from 20° C to 2° C did not suppress the staircase although it did diminish the number of steps, while the ascent of the curve was rather linear. At that temperature, the anoxia from passage of nitrogen during an hour, did not prevent the phenomenon, either, although the systole was then very depressed. However, following 50-60 minutes nitrogen bubbling, the staircase was reduced or abolished (fig. 4).

Electric phenomena of the staircase. It has been demostrated by Hoffmann that the staircase frequently coincides with the reduction of

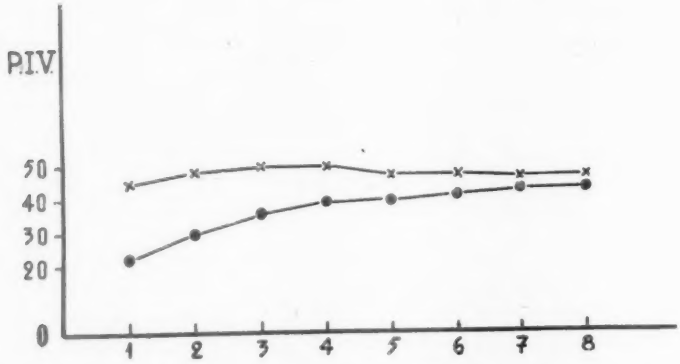


FIG. 3. — Diagram of intraventricular (P I V) in mm Hg developed during a typical staircase *with a diastolic volume of 0.6 cc of Ringer (•——•); likewise, with a diastolic volume of 0.8 cc (×——×). Abscissa: 8 first contractions at a rate of 1 each 2.5 sec. Notice on upper curve the suppression of normal staircase and the negative staircase developing from 5th systole.*

the amplitude and duration of the action potential. Contrarily to this view, Spadolini (1952) has upheld the view that during the occurrence of staircase there takes place in BUFO heart both spacial and temporal summation.

In our experiments, the action potential was examined for several minutes. In most cases, results similar to those shown in figure 5 were observed. Three events are elicitable: a decrease of both R wave voltage and time to peak, as well as of Q-T space. As regards Q and S waves, they appeared very variable and unsuitable for thorough examination. R decline was usually between 0.2 and 0.1 mV per minute. In few experiments did the voltage of this wave increase during the two or three first systoles.

The T wave was the one undergoing the broadest variation, for at an isotonic staircase it became progressively accentuated whereas during an isometric staircase it diminished —at times abruptly— or else became biphasic.

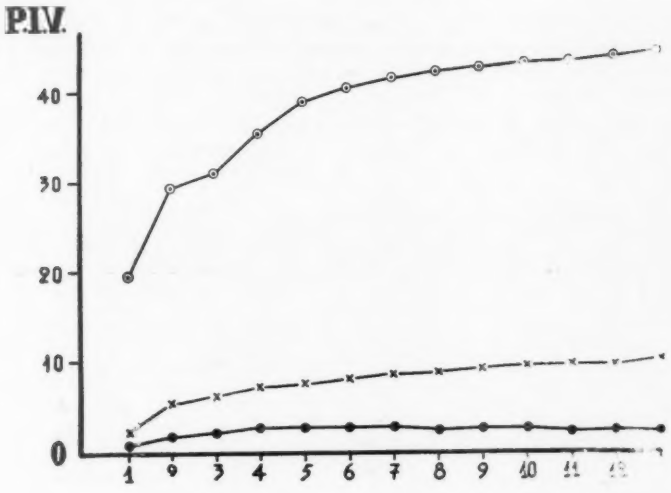
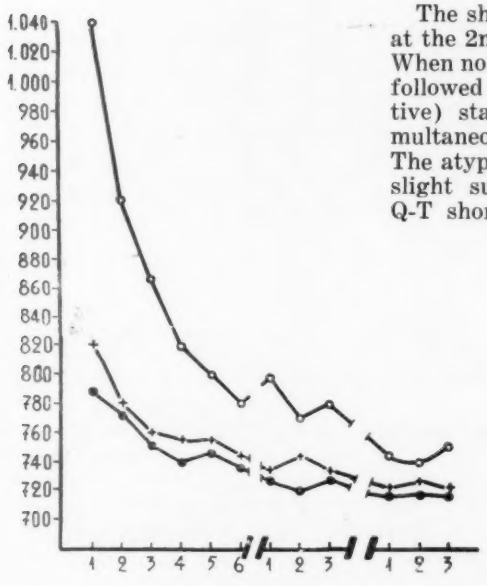


FIG. 4. — Effects of anoxia on staircase. *Intraventricular pressure (in mm Hg) of the first 12 contractions. With oxygen (°——°), 30 minutes after continuous nitrogen bubbling (×——×) and 50 minutes after the passage of this gas (•——•). Observe the intense reduction of the staircase, in the last case.*



The shortening of Q-T was greater at the 2nd systole than at the others. When normal ascending staircase was followed by a descending (or negative) staircase, the time to peak simultaneously diminished with Q-T. The atypical staircase developed with slight successive time to peak and Q-T shortenings.

FIG. 5. — Diagram of space Q-T (°——°), the time to peak (×——×) and of R wave height (•——•), of normal staircase. *Abscissa: 6 first contractions; then, 1 and 2 minutes later. Ordinates: duration of Q-T in ms, duration of time to peak and of R voltage in arbitrary units.*

With the anoxic (from nitrogen addition) staircase the above described electric changes were repeated, just as in the *in situ* heart. When the anoxia persisted, R was of low voltage and did not decrease throughout the series.

The perfusion of the ventricle with Ringer containing $CaCl_2$ in excess (5 mM) greatly diminished or else suppressed the staircase. Under

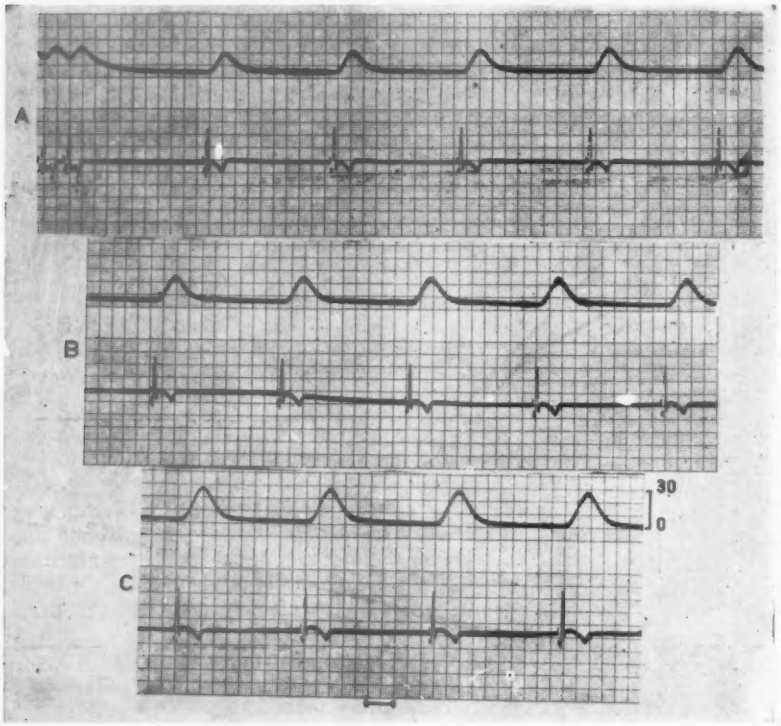


FIG. 6. — Gradual recovery of ventricular inotropism after contractions at a frequency above optimal. *A, B, C: a continuous record of phenomena. Intraventricular presure in mm Hg; electrogram preceded by the stimulation artifact. Inferior edge of the figure 1, second mark. In A, it is possible to observe the end of high frequency contractions followed by the 12 per minute contractions. As the pressure developed by the fibers increases, there is an increase of R height and of Q-T space.*

the above circumstances the formerly described electric modifications, present in the control staircase, were not observed, either.

Cooling modified electrogram pattern. At 5° C the QRS was of little amplitude, with its R undiminished during the staircase, or increasing in heigh in some experiments. This dissociation of electric pattern from

mechanical ones suggest that in these series the contraction had developed gradually through a process diferent from that of normal staircase.

Non optimal stimulation. The rise in stimulation frequency, at the rate of 5-10 per second, during 1-2 minute lapse, provoked increasingly smaller contractions. Intraventricular pressure ranged from 40-50 mm Hg to values of 10-20 mm Hg. Upon re-establishing, without a pause the original optimal frequency, the contractions progressively increased in amplitude; concurrently, there was a rise of voltage for all waves, a QRS shortening and Q-T lengthening (fig. 6). As seen, these changes of electric potential are the reverse of the normal staircase described. These results were likewise observed in the isolated heart activated from stimulated sinusal pacemaker. The gradual increase of R voltage, probably due to a spacial recruitment of fibers, evidences that recovery from the hypodinamic state took place, in those cases, by a mechanism other than that of the normal staircase.

AN ATTEMPT TO A MATHEMATICAL INTERPRETATIVE DESCRIPTION

(by PABLO V. CARLEVARO)

The object of this mathematical study on the increasing staircase of the toad heart muscle is:

1. To give an accurate mathematical description of the quantitative relation between the pressure developed by an isometric contraction of the heart muscle and its temporal location in the set of successive contractions forming the staircase.
2. Furthermore, it was intended to deduce this mathematical expression on the basis of an interpretative formal hypothesis, suited for physico-chemical or physiological studies.

Experimental bases underlying the mathematical description

We are starting from two experimental facts of direct observation:

a) For a given frequency of stimulation, there is a mathematical dependence between maximal pressure H developed at every contraction of the heart and the moment in which the contraction takes place t, measured by the interval of time between the beginning of the staircase and the location in it of the contraction whose maximal pressure is H.

In mathematical terms, we would say that H is a function of t, or:

$$H = F(t) \qquad [1]$$

b) The increasing staircase is characterized by a progressive increase of H as t increases; this growth tends to reach a certain limit value H_0 provided the time of development of the phenomenon is sufficiently prolonged.

Mathematically speaking, we would say that the function [1] is asymptotically increasing, noting:

$$dH/dt > 0, \qquad [2]$$

$$\lim_{t \to \infty} H(t) = H_0 \qquad [3]$$

In practice, the asymptotic value H_0 is reached after a set of con-

tractions, variable in number, at the end of which H attains a constant value with the characteristic range of every biometric value.

Before going any further, we shall make two previous observations. If H is the maximal pressure, its values will only correspond to certain values of t. Hence, H is truly a discontinous function of t which we will treat mathematically disregarding this fact.

The other observation is that there is no stochastic independence between the pairs of values t, H, because none makes sense without the presence of all the previous pairs. This is the whole essence of the staircase and, besides its theoretical importance, we must mention it because the fact that the values are not stochastically independent, does not allow the use of powerful statistical analysis such as the least squares method and the analysis of variance, one of whose bases is the stochastic independence between the values.

Formal hypothetical mechanism of the staircase

Since we have proposed something more than giving a simple empirical equation between H and t —that in this sense does not matter— we have used the above mentioned experimental bases in order to build up a hypothesis which could be expressed in mathematical terms, solved as such and later tested with the experimental findings.

As a first approximation, the simplest assumption that we can make concerning the progressive increase of the maximal pressure developed by the heart muscle, could be expressed by an elementary differential equation such as [4], that takes solution by integration in an exponential function increasing asymptotically [5]:

$$dH/dt = k\,(H_0 - H), \qquad [4]$$

$$H = H_0\,(1 - e^{-kt}). \qquad [5]$$

The equation [4] shows that the growth rate of H, at a given moment of the staircase, is directly proportional to the difference existing between the pressure value attained at this moment: H and the limit value reached asymptotically after a sufficiently long time, k being a proportionality constant the dimensions of which are T^{-1}.

If this hypothesis were in accordance with the experimental data, plotting $\ln (H_0 - H)/H_0$ for any given H, against the corresponding time t, we would have a straight line passing through the origin and whose slope were k; making a logarithmic transformation of [5] we obtain:

$$\ln \frac{H_0 - H}{H_0} = -kt. \qquad [6]$$

The straight-line relationship is not satisfied by the experimental data and therefore, we must think indefensible to consider k as a proportionality constant. This coefficient being variable with time, we shall symbolically write our differential equation of the increasing staircase in the following way:

$$dH/dt = \varphi(t)\;(H_0 - H), \qquad [7]$$

when $\varphi(t)$ symbolizes a certain function of time whose mathematical form can be postulated in different ways.

Assuming a purely mathematical descriptive point of view and without making any assumption on the physico-chemical nature of the factors, we conceived the mechanism of the increasing staircase as the result of the action of two formal factors, of which one predominantly governs the onset of the phenomenon and the other, its prosecution and culmination.

Our theoretical statement establishes that the first factor is initially large and it decreases exponentially in a relatively brief interval of time, being unable by itself to make the staircase reach the maximal level H_0. Nevertheless, it is responsible for the explosive onset of the increasing staircase.

The existence of a second factor growing linearly with time, that is, with the succession of mechanical phenomena, enables the staircase to continue and to reach its own asymptotic value. This factor can give an increasing staircase by itself of the same final value H_0, but in this case the initial slope will be considerably smaller.

According to the previous statement, $\varphi(t)$ will be the sum of two functions of t, each one being in relation with one formal factor, their respective mathematical notations being:

$$\varphi_1(t) = k_0 e^{-\lambda t} \qquad [8]$$

and

$$\varphi_2(t) = \mu t, \qquad [9]$$

where k_0 is the value of the first factor at the beginning of the staircase, when: $t = 0$; λ is in direct relation with the rate of drop of the same factor and μ is the constant rate of growth of the second formal factor.

Their respective dimensions are for k_0 and λ: T^{-1}, and for μ: T^{-2}.

Summing up both functions, $\varphi(t)$ will be:

$$\varphi(t) = k_0 e^{-\lambda t} + \mu t. \qquad [10]$$

Introducing [10] into [7], we find a linear ordinary differential equation of the first order, whose expression is:

$$dH/dt = (k_0 e^{-\lambda t} + \mu t)\ (H_0 - H), \qquad [11]$$

that can be easily solved as a separable equation. This gives:

$$\int \frac{dH}{H_0 - H} = \int (k_0 e^{-\lambda t} + \mu t)\ dt + A, \qquad [12]$$

where A is an arbitrary constant.

Evaluating the integral we obtain:

$$-\ln (H_0 - H) = -\frac{k_0}{\lambda} e^{-\lambda t} + \frac{1}{2}\mu t^2 + A, \qquad [13]$$

when $t = 0$, $H_{t=0} = 0$. Therefore, the value of A will be:

$$A = \frac{k_0}{\lambda} - \ln H_0. \qquad [14]$$

Putting [14] into [13] and making some elementary rearrangements, we find:

$$H = H_0 \left\{ 1 - e^{-\left[\frac{k_0}{\lambda} (1 - e^{-\lambda t}) + \frac{1}{2} \mu t^2 \right]} \right\} \qquad [15]$$

which is the final formula giving the mathematical relation between the maximal pressure H of the contraction that takes place at moment t and this given time.

The parameters H_0, k_0, λ and μ included in this expression have a previously defined meaning and they are typical of both the tissue and its functional state and of the experimental conditions in which the phenomenon was produced.

Discussion of the formal hypothesis

In order to control the agreement between the theoretical formal mechanism assumed and the experimental data, we have calculated by graphical methods the numerical values corresponding to the parameters of our equation [15]. With these values we have drawn the theoretical curve given by [15] which must adjust to its corresponding experimental curve. The adjustment of [15] is shown in figure 1; it is very accurate.

Hence, the accordance between the formal hypothesis and the experimental observation is fairly well satisfied, although we have not been able to discuss it statistically for the previously mentioned reasons.

Besides, this adjustment is equally satisfactory if we fit the function [15] to the so called "atypical" staircase (figure 2), the mathematical description of which me did not intend at first, and in this way we verified that both staircases follow a same mathematical expression and that their differences are only quantitative.

The "atypical" staircases show a decreased explosive factor, k_0 being very small. The second factor prevails quantitatively and this fact conditions the configuration of the recorded staircase.

It is remarkable that, when there are certain quantitative relations between k_0, λ and μ, the function [15] may show two inflection points. This can be observed in the curve (figure 2). The rate of growth of the staircase is slowed down following the initial contractions but it later recovers and finally tends asymptotically towards zero.

The curves shown in the present work represent two extreme varieties of increasing staircases. Formula [15] also describes the intermediate types of these curves. Therefore, disregarding the diversity of the experimental recorded curves, it has been possible to obtain one single interpretation for everyone.

In order to discuss the accuracy of our hypothesis on the description of Szent-Györgyi's ([10]) staircases, we must determine the value of the integration constant A of formula [13] regarding its own experimental conditions. In this case, for $t = 0$, $H_{t=0} = h_0$.

After obtaining A and substituting it in [13], by making some rearrangements this expression will become:

$$H = h_0 + (H_0 - h_0)\left\{1 - e^{-\left[\frac{k_0}{\lambda}(1 - e^{-\lambda t}) + \frac{1}{2}\mu t^2\right]}\right\}. \qquad [16]$$

Fitting the function [16] to the corresponding experimental curves, we find that the adjustment holds equally good.

It is worth noting that only one of the three staircases of the above mentioned author has in its initial explosive factor, an important quantitative value. This explains the similar way in which the other two grow compared with our curve in figure 2.

Szent-Györgyi's ([10]) interpretation of the staircase phenomenon as a consequence of a "favorable condition" created by its own activity is, just like ours, essentially formal. A translation to the language of differential equations would lead to a mathematical expression partly similar to ours.

The "favorable condition" could be formally identified with our second factor, that is, with that growing linearly with time. This factor is clearly associated to the production of tissue activity.

Despite the difference in technique, the experimental conditions for obtaining the phenomenon as well as in the species of animal used, our formula is still valid. We believe this is a strong indication of its formal accuracy.

Discussion

The study of the electrogram during the staircase generally bears out the observations of Hoffmann and those of Trautweinn and Dudel as well as of Niedergerke. Hence, the genuine staircase often coincides with decrease of R voltage and shortening of electric systole. In these cases there is no spacial recruitment but merely temporary action. Nor does it depend on the diastolic length of fibers. Likewise, the phenomenon is not provoked by a gradually improved oxygenation, for it occurs during anoxia nor does it depend on metabolites such as glucose. Hence, these findings substantiate the current criterion by which the staircase is made dependant upon the ions.

In the intact Bufo, the production of staircase under normal circumstances is a likely occurrence. We have seen this gradual recovery of ventricular activity after auriculo-ventricular blocks and after fibrillation (unpublished experiments).

Following repeated contractions, over an optimal frequency, there ensues a gradual recovery to be distinguished from the staircases. As stated earlier, the occurrence of a spacial recruitment in this series is a likely event.

With reference to the mechanism giving rise to the increasing staircase, the mathematical analysis included in this paper distinguished two formal factors. With time, one of them becomes exponentially exhausted, in a rather speedy manner, whereas the other grows linearly with time and becomes indispensable for the staircase to attain its maximum. The first factor causes the explosive initial increase and the second one is

responsible for the continuance of growth until it asymptotically attains its maximal value. This last portion of normal staircase, where the second factor exerts a more conspicuous action, has as yet not been given close consideration. Atypical staircases are of the slow increasing pattern, for in them there prevails, quantitatively, the second factor. However, both staircase patterns and their intermediate variants are described by the same mathematical expression [15].

Recent contributions (Hajdu and Szent-Györgyi) have shown the paramount role of cations during gradual contraction. The factors postulated in the mathematical study of the curve might depend upon the above mentioned ions, thus setting up the activation process required for staircase development.

Summary and conclusions

A study was made of the staircase phenomenon in hearts of Bufo arenarum H. *in vitro* and *in situ*. The persistence of normal staircases in ventricles perfused with whole blood and under conditions of anoxia, was observed. In most experiments the phenomenon was incident to the shortening of electric potential and R wave voltage. These findings frequently permit the differentiation of the staircase phenomenon from other gradual recoveries of the myocardium such as that following excessively fast contractions.

In order to give a purely formal interpretative description to the phenomenon, a mathematical analysis was performed and an ordinary differential equation has been established and solved. This differential equation is the mathematical expression of a formally conceived mechanism of the staircase. According to such mechanism the onset of the phenomenon is governed by a factor that exponentially drops with time which is responsible for the explosive initial enhancement of the series. The staircase reaches asymptotically its maximal value due to a second factor which grows linearly with time. A very suitable agreement between the formal hypothesis and the various experimental patterns was observed. This enables a single formal interpretation for the studied phenomenon.

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TISSULAR DISTRIBUTION AND BILIARY EXCRETION OF THIAMINE ON NEPHRECTOMIZED ANIMALS

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INTRODUCTION

THE CAPACITY of the liver to store thiamine has been studied principally on rats. Leong ([2]) observed that when the thiamine content of the ration was increased from 4 to 25 I.U. (12 to 75 γ) per gm, the thiamine level of the liver did not change (2.6 I.U. or 7.8 γ per gm of tissue). Identical results are reported by Sure and Ford ([6]).

In the present publication we demonstrate that when thiamine is injected in nefrectomized animals, the liver concentrates the vitamin above the plasmatic levels, even when high dosis are employed. It was also observed that large amounts of thiamine are excreted in the bile.

MATERIAL AND METHODS

Thiamine was determined by the microbiological assay of Sarret and Cheldelin ([5]) with slight modifications. Volumes of 0.2 to 10 ml of liquid samples (plasma, bile, pancreatic and salivary secretions) were diluted to 40 ml with 0.1 N sulfuric acid. Tissues were ground with sand before dilution. These acidified samples were steamed for half hour and, after adjusting the pH to 4.5 and completing the volume to 50 ml, steamed again for 15 minutes and filtered while hot. The filtrates were adjusted to pH 6.5 with a few drops of 10 % NaOH, steamed for 15 minutes,

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filtered and diluted to contain approximately 20 mγ of thiamine per ml. The assays were set with a standard curve from 0 to 100 mγ of thiamine HCl, incubated for 18 hours at 37° C and read turbidimetricaly on the Evelyn photoeletric colorimeter with filter 620. The basal medium was prepared with peptone instead of yeast extract; 40 gm of "DIFCO" peptone were dissolved in 250 ml of hot water; after cooling, the solution was mixed with 250 ml of 2 N NaOH and left for 24 hours in the refrigerator. The pH was adjusted to 6.5 with glacial acetic acid, 7 gm of anh. sodium acetate were added and the preparation was autoclaved for 15 minutes at 15 pounds. It was finaly diluted to contain 65 mg of peptone per ml and used at the proportion of 1.5 ml per tube.

The total liver fat was determined by the method of Van De Kamer *et al* (7). One to 3 gm of liver were cut to small pieces, refluxed for half hour with 60 % KOH, acidified and extracted with petroleum ether. The extract was titrated with KOH in isobutanol, using a solution of thymol blue in isobutanol as indicator.

The samples for thiamine and fat determinations were stored at − 10° C until analysed.

Nembutal (40 mg per kg of body weight) was injected in the vein (dogs) and in the peritoneum (rats). Urethane (2.8 gm per kg of body weight) was injected at the proportion of 60 % in the muscle and 40 % in the peritoneum. The animals used were albino male rats, Wistar strain, and male adult mongrel dogs. All the nefrectomies were bilateral.

Results

1. *Distribution of thiamine in the tissues of nephrectomized dogs*

Thiamine determinations were carried in the blood and tissues of two dogs nephrectomized under nembutal anesthesia, injected with thiamine intravenously and bled to death one hour after thiamine administration. The results (table I) demonstrate that the liver is the only tissue to present a thiamine content above the plasma.

This capacity of the liver to concentrate thiamine was further investigated in another experiment. A nephrectomized dog was injected intravenously with increasing amounts of the vitamin. Blood samples and liver biopsies were obtained 20 minutes after each injection. The results are summarized in table II. On this table the capacity of the liver to concentrate thiamine above the plasma is indicated by the ratio *liver thiamine / plasma thiamine*. Although this ratio decreases when increasing amounts of the vitamin are administrated, approximately 50 % of the thiamine injected is retained by the liver, as may be seen from the right column on table II.

2. *Distribution of thiamine in the liver and plasma of nephrectomized animals*

In order to investigate if the capacity to concentrate thiamine is also present in the liver of the rat, 7 animals were nephrectomized under nembutal anesthesia and injected intravenously with increasing amounts of thiamine. Thirty minutes after the injection, blood and liver samples were collected for thiamine determination. The results are presented in table III. They show that the liver content is above the plasmatic

TABLE I

Thiamine levels on plasma, red cells and tissues of nephrectomized dogs, one hour after intravenous thiamine administration

	Thiamine in γ/gm or ml	
	Dog 14-12 kg (10 mg of B_1/kg of body weight)	Dog 13-10 kg (40 mg of B_1/kg of body weight)
Plasma	16.6	264.3
Red cells	3.9	15.0
Tissues		
Liver	44.0	568.0
Brain	2.2	6.8
Cerebellum	2.5	7.4
Medulla oblongata	2.3	7.5
Lung	10.4	44.0
Heart	10.0	166.0
Spleen	4.8	80.0
Stomach	10.5	153.0
Small intestine	4.7	76.0
Large intestine	3.8	90.6
Submaxillary gland	8.6	70.0
Pancreas	13.8	45.0
Hypophysis	7.8	55.0
Adrenals	7.1	66.0
Thyroid	8.3	67.0
Testes	4.5	70.0
Skeletal muscle	3.3	35.0
Skin	7.7	10.0

level, even when the highest dosis tolerated by the animal —54,6 mg—, is injected.

This capacity of the liver to maintain thiamine concentrations above the plasma is still present 40 hours after the injection, as is brought to evidence by the experiment summarized in table IV. Six rats were nephrectomized asepticaly under ether anesthesia and 4.5 mg of thiamine per kg of body weight injected intravenously. At different times after the injection, the animals were again etherized, and samples of plasma and liver collected for thiamine determination.

3. *Excretion of thiamine in the bile*

The observation that the liver concentrates thiamine above the plasmatic levels led us to investigate the possibility of thiamine excretion through the bile. Salivary and pancreatic secretions were also studied for comparison. Three dogs under nembutal anesthesia were submitted

TABLE II

Thiamine levels on plasma and liver of a nephrectomized dog injected intravenously with increasing amounts of thiamine. Dog 15, 12 kg. Weight of the liver at the end of the experiment, 264 g.

Thiamine injected (mg)	Plasma thiamine (γ/ml)	Liver thiamine (γ/gm)	Liver thiamine / Plasma thiamine	Thiamine retained by the liver: mg	Thiamine retained by the liver: As % of the injected
—	0.049	2.1	42.8	—	—
6	1.2	11.9	9.9	3.1	52
18	6.0	34.0	5.7	9.0	50
54	18.6	92.1	5.0	25.2	47
162	75.0	285.7	3.8	75.4	47

to nefrectomy and colecystectomy. The biliary, submaxilary and pancreatic ducts were cannulated with polyethylene tubes. The pancreatic secretion was stimulated at the time of collection with secretin *. For stimulation of the salivary secretion, 1 mg of pillocarpine was injected intravenously. Bile was collected without the use of stimulants. Samples of bile, salivary and pancreatic secretions and liver biopsies were obtained before, and 30 and 210 minutes after thiamine administration.

TABLE III

Thiamine levels on plasma and liver of nefrectomized rats, 30 minutes after administration of different dosis of thiamine

Rat Nº	Weight (gm)	Thiamine injected (mg/kg body weight)	Liver thiamine (γ/gm)	Plasma thiamine (γ/ml)
1	139	0.5	5.6	0.65
2	149	1.5	17.0	1.3
3	151	4.5	35.0	4.5
4	156	13.5	75.4	14.0
5	156	40.5	309.0	126.0
6	161	121.5	565.0	214.0
7	192	285.0 *	1234.0	873.0

* Injected very slowly.

* Secretin "Wyeth", on the basis of 1 dog unit per kg of body weight.

The results are presented in table V. They demonstrate that the thiamine levels of the bile are higher than on the plasma, at least when reasonably high dosis of thiamine are injected. Pancreatic and salivary secretions exhibit only small increases on their thiamine contents and are below the plasmatic levels in all determinations.

TABLE IV

Thiamine levels on plasma and liver of nephrectomized rats, injected intravenously with 4.5 mg of thiamine / kg of body weight and studied at different times after the injection

Rat Nº	Weight (gm)	Period of observation (minutes)	Liver thiamine (γ/gm)	Plasma thiamine (γ/ml)
1	205	10	57.0	12.6
2	251	30	72.0	7.0
3	220	90	46.0	4.6
4	270	270	49.0	3.4
5	232	810	49.0	0.86
6	278	2.430	30.1	0.98

The thiamine excretion through the bile was also investigated in rats. Seven animals were nephrectomized under nembutal anesthesia and the biliary duct cannulated close to the liver with polyethylene tubing. Thiamine —35 mg per kg of body weight— was injected intravenously. The bile was collected during 2 hours, in 5 ml graduated cylinders placed in icy water and protected from the light. Bile, liver and plasma were saved for thiamine assay. The results are presented in table VI. It is evident that the rat has also the capacity to excrete thiamine in the bile at higher concentrations than those present in plasma.

4. *The influence of liver injury on the biliary thiamine excretion*

It was found interesting to investigate if the liver injury produced by carbontetrachloride affects the capacity of the liver to concentrate thiamine above the plasmatic level. Twelve rats caged individualy received *ad libitum* the following ration: casein, 20 % ; peanut oil, 10 % ; saccharose, 66 % ; salt mixture IV [Phillips and Hart ([2])], 4 %. The following vitamin supplement was added per kg of ration: thiamine, riboflavin, pyridoxin, calcium pantothenate, para-aminobenzoic acid and vitamin K, 5 mg each; niacin, 10 mg; folic acid, 1 mg; d-biotin, 0.05 mg; choline, 1 mg; inositol, 100 mg. Once a week, 1.000 I.U. of vitamin A, 300 I.U. of vitamin D and 5 mg of alphatocopherol were given by mouth. Six rats received daily during 14 days 0.1 ml of carbon tetrachloride by subcutaneous route; the others were kept as controls. At the 15th day on experiment control and injected animals were nephrectomized under urethane

TABLE V

Thiamine levels on plasma, liver, bile, pancreatic and salivary secretions of nephrectomized dogs before and after intravenous thiamine administration

Dog Nº and weight	Thiamine injected (mg/kg of body weight)	Period of observation	Thiamine in γ/gm or ml				
			Plasma	Liver	Bile	Pancreatic secretion	Salivary secretion
10 (22 kg)	0.5	before	0.034	1.7	0.040	0.074	0.027
		30 min after B_1	1.0	9.7	4.9	0.050	0.012
		210 min after B_1	0.22	4.5	0.10	0.016	0.019
11 (16 kg)	1.5	before	0.023	1.2	0.00	0.021	0.016
		30 min after B_1	3.1	16.8	18.0	0.14	0.058
		210 min after B_1	1.7	11.0	0.55	0.18	0.052
12 (18 kg)	4.5	before	0.018	2.5	0.004	0.107	0.022
		30 min after B_1	9.1	92.5	75.7	1.3	0.45
		210 min after B_1	6.3	53.5	31.1	0.59	0.15

anesthesia and the biliary duct cannulated close to be liver. 35 mg of thiamine per kg of body weight were injected intravenously and the bile collected during 2 hours as previously described. At the end of the experiment samples of blood and liver were collected for thiamine and fat determinations. The results are presented in table VII. The capacity of the liver to concentrate thiamine above the plasmatic levels was

TABLE VI

Thiamine levels on plasma, bile and liver of nephrectomized rats injected with 35 mg of thiamine/kg of body weight. Period of observation, 2 hours

Rat Nº	Bile volume (ml)	Bile thiamine (γ/ml)	Liver thiamine (γ/gm)	Plasma thiamine (γ/ml)
1	0.5	97	—	47
2	1.0	255	—	57
3	1.2	198	—	114
4	1.0	171	—	72
5	0.8	365	231	—
6	0.75	112	298	—
7	0.80	115	294	—

identical in both groups. Although the average thiamine content of the bile was lower in the CCl_4 injected group, the interpretation is complicated by the fact that the body weight of the intoxicated animals at the end of the experiment was 32 % lower than the body weight of the controls and consequently they received 32 % less thiamine.

Discussion

The mechanism of thiamine storage by the liver of nephrectomized animals is unknown. Preliminary studies indicate that this thiamine is not phosphorilated. The results presented in tables IV and V indicate that it has a tendency to decrease after the injection. This cannot be explained by the biliary excretion. For example, on dog 12, table VI, the liver thiamine concentration was 92 γ/gm thirty minutes after the injection and 50.5 γ/gm at the end of the experiment. As the weight of the liver at the end of the experiment was 440 gm, the reduction on the total thiamine content of the organ was 18.260 mg. The total bile flow during this period was 17 ml. An average thiamine concentration of 1 mg per ml in the bile would be necessary to explain this reduction on the total thiamine content of the liver. Sure and Ford (6) demonstrated that there is an appreciable destruction of thiamine when it is incubated

TABLE VII

Liver, biliary and plasmatic thiamine and liver fat on control and on CCl_4 intoxicated rats

Group	Rat Nº	Liver thiamine (γ/gm)	Bile thiamine (γ/ml)	Bile volume (ml)	Plasma thiamine (γ/ml)	Liver fat (gm %)
Control	1	118	48	0.5	38	9.2
	2	201	84	0.8	32	4.5
	3	125	140	1.0	16	—
	4	133	134	0.9		3.8
	5	175	244	0.7		4.7
	6	153	97	0.5		5.1
Averages		161	124.5	0.75	29	5.5
CCl_4 intoxicated	7	155	62	0.8	17	9.5
	8	207	44	0.8	28	9.2
	9	80	52	0.6	35	22.1
	10	105	144	1.0		6.7
	11	175	67	0.4		16.4
	12	127	60	0.3		13.8
Averages		141	71.5	0.65	27	12.9

with the liver and other tissues of the rat and suggested that part of the losses of thiamine in the metabolism are caused by this destruction in the tissues. This mechanism would explain the decrease on the thiamine level observed on rats (table V) and dogs (table VI) on the present experiments.

It has been claimed that the liver injury reduces the thiamine storage capacity [Borson (1), Williams and Bissell (8), Williams *et al* (9)]. However we could not find a reduction of the capacity of the liver to concentrate thiamine above the plasmatic levels when CCl_4 intoxicated rats were nefrectomized and injected with the vitamin.

Salcedo *et al* (4) demonstrated that the central nervous system maintains its normal thiamine concentration for longer periods than other tissues (heart, liver and kidney) and concluded that the brain differs from other tissues in not being able to store surplus thiamine. Our data (table I) support their statement, suggesting that the central nervous system has a very low permeability for thiamine.

SUMMARY

1. When thiamine is injected in nephrectomized dogs (10 mg and 40 mg/kg of body weight) all the tissues have lower concentrations than the plasma, with exception of the liver, that exhibits much higher levels.

2. This capacity of the liver to concentrate thiamine above the plasmatic levels is observed on dogs and rats even when the amounts of vitamin injected are very high.

3. The bile of nephrectomized and thiamine injected animals has higher thiamine concentrations than the plasma (rats and dogs) but this is not observed in pancreatic and salivary secretions (dogs).

4. Liver injury, as produced by carbon tetrachloride does not interferes with the capacity of the liver to concentrate thiamine (rats).

SUMARIO

1. Quando se injeta tiamina em cães nefrectomisados (10 mg e 40 mg por kg de peso), todos os tecidos apresentam concentrações mais baixas que a do plasma com exceção do fígado, em que os níveis são mais elevados.

2. Esta capacidade do fígado de concentrar tiamina acima dos níveis plasmáticos é observada tanto em cães como em ratos mesmo quando as quantidades de tiamina injetadas são muito altas.

3. A concentração de tiamina na bile de cães e ratos nefrectomisados e injetados com esta vitamina é mais alta que a do plasma, fato este que não se observa nas secreções salivar e pancreática.

4. A lesão hepática produzida por tetracloreto de carbono não diminue apreciavelmente a capacidade do fígado de concentrar tiamina em níveis superiores aos do plasma.

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SODIUM CHLORIDE AND THE PRESSOR EFFECT OF THE GONADOTROPHINS

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IT HAS BEEN shown that gonadotrophin administration (serum and chorionic) produces an evident pressor effect in male rats with renal reduction. However, this pressor effect did not appear in females (1).

In the present study the effect of the associate administration of gonadotrophins and 1 % sodium chloride, given as drinking fluid, was observed in male and female rats, castrated and non castrated ***.

MATERIAL AND METHODS

Albino rats weighing 200-300 g were used. They were fed normal diet; 1 % sodium chloride ad libitum substituted tap water in experiments which will be singed. All the animals were submited to the same renal reduction. It consisted of a ligature in 8 of the left kidney, followed one week after by the right nephrectomy. (Grollman's technique, 1944). In two groups bilateral adrenalectomy was performed in two operations, at same time of the operations on the kidneys. Gonadectomy in males and females was performed by usual technique one week prior the beginning of the renal reduction. The blood pressure was determined, with maximal intervals of five days, by the technique of Williams, Harrison and Grollman (1939), slightly modified (2). Equal quantities of serum (1) and chorionic (2) gonadotrophins were dissolved in saline solution (0.9 %) and injected subcutaneosly, 10 U/rat/day.

RESULTS

A. — *Effect of 1 % NaCl 40 days after gonadotrophin injection.*

Two groups of 8 male and 9 female rats were injected gonadotrophins during 40 days starting immediately after the renal reduction. At the

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*** 1, gonadotrophil; 2, gonacoryl. (Kindly supplied by the Laboratories Silva Araujo Roussel S. A., Brazil).

TABLE I

Blood pressure evolution of the rats with renal reduction receiving H_2O or 1 % NaCl

S	N initial	DRINKING FLUID	BLOOD PRESSURE (WEEKS)		
			2nd	4th	6th
♂	15	NaCl	6a - 4b - — / 15	6a - 4b - 1c / 12	4a - 6b - 1c / 11
♂ C	6	NaCl	5a - — - — / 6	1a - 2b - — / 4	2a - — - 2c / 4
♂	6	H_2O	6a - — - — / 6	6a - — - — / 6	6a - — - — / 6
♀	10	NaCl	7a - — - 1c / 10	5a - 1b - 3c / 9	5a - — - 1c (3) * / 6 (8)
♀ C	6	NaCl	5a - — - — / 6	3a - 1b - 2c / 6	3a - — - 2c / 5
♀	6	H_2O	4a - — - — / 6	4a - — - — / 6	3a - — - — / 6

a: 130-150 mm Hg; b: 150-170 mm Hg; c: > 170 mm Hg.

* 2 rats with blood pressure higher than 170 mm Hg (c) died a few days before the 6th week.

C, castrated.

end of this time 5 out of 8 males presented a moderate hypertensión (130-150 mmHg). However, all the 9 females were normotensives. This sexual difference in the pressor effect of the gonadotrophins corroborates the published results (1). After this first period the animals were given gonadotrophins for another periiod of 40 days in which 5 females and 4 males received 1 % sodium chloride instead of water.

At the end of the experiment all males with 1 % sodium chloride became hypertensives, and the same happens with 3 out of 4 of those receiving water. There was not great difference between hypertensive values of these rats having sodium chloride and those having water. In the females, only 1 out of 4 receiving water was hypertensive, but 4 out of 5 with 1 % sodium chloride presented a high blood pressure (> 170 mmHg).

B. — *NaCl and NaCl + gonadotrophins immediately after the renal reduction.*

In order to further clarify the data obtained in the previous series some other experiments were performed.

At first the blood pressure alteration immediately following the renal reduction was studied in our rats, as well as, the modification

produced by the administration of 1 % sodium chloride. The sodium chloride had an evidend pressor effect (Table I). This effect was approximately the same either in males or females. Gonadectomized male and female animals, presented the same response.

Only in non castrated male rats, the pressor effect of gonadotrophins + NaCl was greater than the pressor effect of the 1 % sodium chloride

TABLE II

Blood pressure evolution of the rats with renal reduction receiving gonadotrophins + 1 % NaCl

S	N initial	BLOOD PRESSURE (WEEKS)		
		2nd	4th	6th
♂	10	3a - 4b - 2c / 10	1a - 1b - 5c / 7	1a - — - 6c / 7
♂C	10	1a - 5b - 2c / 10	6a - 1b - 2c / 9	3a - 1b - 2c / 6
♀	13	3a - 4b - 3c / 13	4a - 5b - 2c / 11	2a - 3b - 4c / 9
♀ C	12	4a - 2b - 1c / 12	5a - 2b - 2c / 11	2a - 2b - 4c / 11

a: 130-150 mm Hg; b: 150-170 mm Hg; c: > 170 mm Hg.
C, castrated.

alone (Table II). In castrated males and in the females, ovariectomized or not, receiving gonadotrophins + NaCl the blood pressure values were aproximately the same as in those receiving only 1 % sodium chloride.

C. — *Gonadotrophins + NaCl in adrenalectomized rats.*

It is useful to remember that previously, it was observed that our rats had accesory adrenals and they not need additional sodium chloride to survive 15 days after the total adrenalectomy.

Two groups of 20 males and 14 females received gonadotrophins and 1 % NaCl immediately after the renal reduction operations and concomitant bilateral adrenalectomy. The survival was very poor in both groups, at the end of the fourth week only 20 % of the females and 50% of the males were alive. Until the second week there was not development of hypertension neither in males nor in females Table III). However, at the fourth week the blood pressure in these groups was not essentially different from that of the animals in which the adrenalectomy was not

TABLE III

Blood pressure evolution of the rats with renal reduction and adrenalectomized, receiving gonadotrophins + 1 % NaCl

S	N initial	BLOOD PRESSURE (WEEKS)	
		2nd	4th
♂	20	1a - — - — / 13	4a - 1b - 3c / 11
♀	14	1a - — - — / 7	1a - 1b - 1c / 3

a: 130-150 mm Hg; b: 150-170 mm Hg; c: > 170 mm Hg.

performed (Table II). In a few adrenalectomized males the treatment was continued until the 11th week, with no great modification in the blood pressure values observed at the 4th week.

Discussion

Daily injection of gonadotrophins during 40 days after the renal reduction in rats drinking water, determinated hypertensión only in males. This is in agreement with the published results (1). The continuance of gonadotrophin injection in this animals for 40 days more, and the substitution of tap water for 1 % sodium chloride as drinking fluid, caused a severe hypertension in the females. In this series the pressor effect of the sodium chloride on the males was not so evident.

In the experiment B gonadotrophins + NaCl or NaCl alone were administrated immediately after the renal reduction. The development of hypertension in the females, ovariectomized or not, was practically the same when receiving gonadotrophins + NaCl or NaCl alone. This is another proof in the sense that gonadotrophins have not pressor effects in female rats, and also suggests that hypertension which appears in the females of the experiment A may be attributed to the pressor effect of the sodium chloride alone.

In males with renal reduction, only in those which were not castrated, the pressor effect of gonadotrophins + NaCl was greater than the pressor effect produced by each one of these agents when acting isolatedly. This indicates that for the appearance of the pressor effect of gonadotrophins testis are necessary.

Two weeks after the adrenalectomy the rats survived without additional sodium chloride. It can be assumed that as this time they had a good adrenal function. However, there are not elements to determine the degree of adrenal insufficiency during the first two weeks. It is

interesting that the adrenalectomized rats receiving gonadotrophins and 1 % sodium chloride did not develop hypertension until the second week as did the non adrenalectomized. At the 4th week the blood pressure in these animals was approximately the same as in those non adrenalectomized. It was observed that two weeks after the adrenalectomy, in rats which develop accesory adrenals, the blood pressure was normal and the some time later, was greater than the normal values ([3]). More recently hypertension was described in rats unilaterally adrenalectomized and with contra-lateral adrenal enucleation ([4]); this rats were also unilaterally nephrectomized and received sodium chloride 1 % ad libitum. A few of our male adrenalectomized rats receiving gonadotrophins and 1 % sodium chloride were observed until the 11th week. Their blood pressure was not significantly different from that of the group not adrenalectomized.

Summary

Gonadotrophins (10 U/rat/day) injected in rats with renal reduction (ligature in 8 of the left kidney and right nephrectomy) and drinking 1 % sodium chloride had pressor effects only in the non castrated males. This effect was not observed in castrated males nor in females, ovariectomized or not. Adrenalectomy did not prevent this effect after the second week.

Acknowledgements

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THE EFFECT OF INSULIN ON THE ELECTRICAL ACTIVITY OF ISOLATED VENTRICULAR MUSCLE OF THE RAT *

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INSULIN is able to influence the metabolism of muscle (1) and also, as has recently been shown, the resting potential of skeletal muscle fiber (2). Although it has been demonstrated that insulin causes an alteration in the electrocardiogram of dogs (see bibliography in 3) we are not aware of any attempts to determine the influence of this hormone on transmembrane potentials of cardiac muscle. In the present report, the effects of insulin administration on the resting and action potentials of isolated rat ventricular muscle is described.

MATERIAL AND METHODS

White rats of the Sherman strain weighing approximately 200 grams were used. The heart was rapidly removed and immersed in an oxygenated modified Tyrode solution of the following composition: (concentrations given in mM/liter) Na Cl 137, NaHCO3 12,K Cl 2.7, Mg Cl 0.5, Na H PO4 1.8, Ca $Cl_2$2.7, dextrose 5.5. The left ventricle was oponed with sharp scissors and a papillary muscle or a ventricular column carefully removed, transferred to a specially designed chamber and perfused with the modified Tyrode solution. The muscle was stimulated at constante rate of 120/minute and the temperature of the bath kept at 39 $\pm$ 1°C. Electrical activity was recorded from surface fibers of the ventricular strip with microneedles having an outside diameter of less than 1 μ, filled with 3M K Cl using a cathode follower, amplifiers, an oscilloscope etc. as previously described (4). After allowing 15-20 minutes for equilibration, 10 to 30 control recordings were made and the cardiac muscle then perfused with Tyrode solution containing 0.2 U of crystalline Insulin/ml.

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15 to 20 minutes were permitted for equilibration under these conditions and 10 to 30 measurements of resting and action potentials were then made. Finally the muscle was again perfused with the original insulin-free solution and further records were taken after 15 to 20 minutes *.

The records obtained were enlarged and the following parameters measured: 1) Resting potential, 2) overshoot of the action potential, 3) time to half repolarization and 4) time to three-quarter repolarization. The method of making this measurement is shown in Figure 1. These

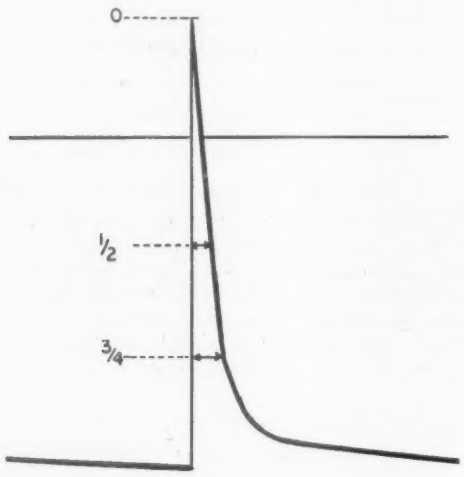

FIG. 1. — *Cellular action potential. Method used in measuring one-half and three-quarter repolarization time.*

latter two measurements were chosen arbitrarily merely in order to estimate the duration of the action potential and the speed of the repolarization process.

RESULTS

1) *Resting potential and overshoot.* No changes in resting potential or in overshoot were seen either when insulin was added to the perfusion fluid or when the ventricular strip was returned to the insulin-free liquid (Table 1).

2) *Duration of the action potential.* The duration of the action potential, as determined by measuring time to the one-half and three-

* In one instance the phenol (0.2×10^{-5} g) used as preservative in insulin, was added to the perfusing fluid without affecting the transmembrane potentials.

TABLE I

Effect of insulin on transmembrane potentials of ventricular muscle of the rat

Exp.	Resting potential (mV)			Overshoot (mV)		
	Tyrode (1)	Insulin (0,2 U/ml)	Tyrode (2)	Tyrode (1)	Insulin (0,2 U/ml)	Tyrode (2)
	•	•	•	•	•	•
12-9 Nº 1	(15) 65.6 ± 5.91	(14) 66.8 ± 9.75	(11) 65.2 ± 3.85	(13) 24.0 ± 6.56	(13) 25.7 ± 7.0	(10) 27.9 ± 5.54
12-9 Nº 2	(12) 66.1 ± 6.64	(13) 65.3 ± 3.30	(14) 68.6 ± 5.82	(12) 21.7 ± 7.37	(13) 11.0 ± 4.58	(14) 14.1 ± 3.90
12-10		(9) 72.6 ± 7.99	(10) 71.0 ± 5.52		(9) 22.2 ± 7.07	(10) 23.4 ± 7.54
12-17	(12) 67.4 ± 4.22	(13) 66.8 ± 4.65	(17) 67.0 ± 4.25	(13) 18.1 ± 7.55	(12) 20.5 ± 7.61	(17) 22.6 ± 5.87
12-24	(29) 61.9 ± 5.99	(17) 61.4 ± 6.89		(25) 11.6 ± 6.20	(17) 12.7 ± 3.20	

	½ repolarization (msec)			¾ repolarization (msec)		
	Tyrode (1)	Insulin (0.2 U/ml)	Tyrode (2)	Tyrode (1)	Insulin (0.2 U/ml)	Tyrode (2)
	•	•	•	•	•	•
	(16) 15.5 ± 1.41**	(15) 12.0 ± 1.41++	(11) 14.5 ± 1.09	(16) 23.9 ± 3.75**	(14) 16.8 ± 1.27++	(11) 20.5 ± 1.70×
	(12) 11.5 ± 3.0**	(13) 6.1 ± 1.08++	(14) 9.8 ± 2.53	(12) 16.0 ± 4.12**	(13) 10.3 ± 1.64++	(13) 15.2 ± 1.53
		(9) 8.0 ± 2.73+	(10) 11.9 ± 2.64		(9) 13.2 ± 1.41++	(10) 20.1 ± 3.50
	(13) 7.8 ± 2.23	(13) 7.7 ± 2.51+	(17) 10.6 ± 2.12×	(13) 11.7 ± 9.16	(13) 12.0 ± 3.46++	(17) 17.5 ± 2.75
	(29) 3.6 ± 0.97	(17) 4.8 ± 1.47		(27) 5.9 ± 1.03	(17) 8.4 ± 1.45	

• Number of penetrations.
* Difference tyrode (1) — insulin significant at the level of $p < 0.01$.
** Difference tyrode (1) — insulin significant at the level of $p < 0.001$.
++ Difference insulin — tyrode (2) significant at the level of $p < 0.01$.
+ Difference insulin — tyrode (2) significant at the level of $p < 0.001$.
× Difference tyrode (1) — tyrode (2) significant at the level of $p < 0.01$.

quarter repolarization was shortened by insulin in 2 out of 4 experiments; the two hearts in which this effect was not seen had very short action potential from the start. When after the insulin produced effect had been observed the ventricular muscle was again perfused with an insulin-free solution, duration of the action potential always increased.

3) *Variations between control experiments.* In order to determine variations of tissue state under conditions established as a control, comparisons were made of ventricular muscle action potentials while being with the modified Tyrode solution alone, before and after having been exposed to action of insulin. No difference was seen in resting potential or overshoot of the action potential. In one instance the repolarization time was longer and in another it was shorter in the post-insulin control period than it was in the pre-insulin period (Table 1 C). Variations observed while exposed to insulin were of a significantly greater magnitude.

Discussion

No changes in resting potential nor in the overshoot of action potential of rat ventricular muscle were produced by insulin. Contrary findings have been reported by Zierler, namely, an increased resting potential for the skeletal muscle of the rat. Reasons for this discrepancy are not apparent. The hormone, though, was able to speed up the repolarization process in ventricular muscle unless the duration of the action potential was already very short. This modification of the repolarization process may explain the changes effected by insulin on the T wave of the electrocardiogram in dogs ([3]). Mechanisms involved in the production of this effect have not been determined, but it is possible that insulin may increase K^+ outflow or Na^+ outflow during repolarization, modify cell membrane permeability or intracellular metabolism or finally modify a variable combination of the aforementioned processes.

Summary

The effect of insulin on the isolated ventricular muscle of the rat has been studied. No changes in the resting potential nor in the overshoot of the action potential were seen but the duration of the repolarization process was shortened.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

April 10, 1958

Relationship between blood viscosity and concentration, size and form of erythrocytes. G. RATHE. *(Instituto de Biología de la Altura, S. S. de Jujuy, Argentina).*

Viscosity of human, ox, cheep and ben blood has been determined with the Ostwald viscosimeter.

The relationship between viscosity and hematocrit values can be represented by the equation:

$$\eta_c = \frac{\eta_0}{0.9669 - 0.0126\ H}.$$

The size and shape of the red cells did not show any influence on the blood viscosity.

Effect of hypoglycemic sulphonamides in the "Bufo arenarum" Hensel. B. A. HOUSSAY AND J. C. PENHOS. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

Normal toads treated with BZ 55, D 860, 2254 RP or 2259 RP showed a clear hypoglycemia between the third and the fifth hour after administration.

The sensitivity of toads to sulfodrugs was in order of intensity, maximal in the adrenalectomized, medium in hypophysectomized and lower in normals.

No significant change in the glycemic level of toads deprived of pancreas, adrenals and pancreas and liver and pancreas was observed when the animals were given BZ 55.

Treated toads, deprived of the liver, showed on the third hour, a significant fall of the glycemic level.

Pharmacology of phenethyl-diguanide. A. ASHKAR, C. N. BURRIER AND M. C. DE PERALTA RAMOS. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Paraguay 2155, Buenos Aires).*

1) This substance had a bi-phasic action on the pressure: a variable inital, very short, ascension, followed by a hypotension, slow and prolonged, increasing with the dose. When animals are given toxic dosis death occurs through hypotension.

2) Heart frequency decreased, but not after bilateral vagotomy. With high dosis, the inhibiting action of the vagus on the heart is diminished. The hipotensive action of acetilcholine could be lowered with high dosis.

3) The hypertensive action of adrenaline (10 μg intravenous) was diminished after the intravenous injection of 25 to 50 mg/kg of DBI (intravenous) and went down to 20 % of its value with 74 mg/kg of DBI. Once it was suppressed, the hypertension produced by stimulation of splanchnic major nerve was diminished (36 mg/kg) or suppressed (74 mg/kg of DBI).

The hypertensive action of nicotine (1 mg intravenous) decreased after 10 mg/kg of DBI and was suppressed with dosis of 22 mg.kg or more. Even 5 and 10 mg of nicotine did not produce any hypertension, but polypnea was obtained.

4) DBI decreased the hypertensive action of 10 mg of Barium chloride.

5) No suppression of the contraction of the nictitant membrane by exciting the pre and post ganglionic fibers of the superior cervical sympathetic ganglion.

6) DBI caused a pupilary dilation which increased with dosage and was reversible when the quantity was not high. The already dilated pupil dilated still more on the cervical sympathetic nerve being excited.

7) The paralysing action of the sympathetic and the sympathetic-mimetical nerves was stronger in the vessels, moderate in the pupils and could not be noted in the nictitant membrane.

8) DBI provoked a relaxation of little intensity of the isolated intestine and uterus of the rat and there was partial antagonism through the action of Barium chloride and acetilcoline.

9) The toxic effects were observed under much bigger dosis than therapeutic ones and with intravenous injections, that are much more efficient than the oral, the latter being the only manner of administering DBI to human beings.

Toxicity of phenethyl-diguanide (DBI). J. C. PENHOS AND J. R. BLAQUIER. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

The toxic symptoms of N'-beta-phenethyl-formamidinyliminourea (DBI, PEDG or PFU) were studied, determining the lethal dose 50 in dogs (oral, 50 mg/kg; subcutaneous, 37.5 mg/kg), rats (oral, 800 mg/kg; subcutaneous, 100 mg/kg), guinea pigs (oral, 37.5 mg/kg), cats (oral, 50 mg/kg) and rabbits (oral, 100 mg-kg).

Hypophysectomized dogs and adrenalectomized dogs and rats were more sensitive to DBI than normals.

Adrenaline, glucose and hydrocortisone had a protective effect on the mortality of rats, but not on the mortality of dogs, according to the dosis employed.

Toxicity is due mainly to hypotension and to the action on the nervous system (Ashkar, Bunier and Cattaneo, 1958). Hypoglycemia can be an additional factor (in rats) to these toxic effects.

No changes were observed in the blood sugar level of pancreatectomized dogs and mortality was lower than in the normals.

May 5, 1958

Role of the thyroid on the eosinopenia produced by piribenzamine in the white rat. H. CHIODI AND J. L. SCARO. *(Instituto de Biología de la Altura, S. S. de Jujuy, Argentina).*

Effects of piribenzamine on circulating eosinophils of thyroidectomized rats or rats with spontaneous goiter have been studied.

Thyroidectomy prevented the eosinopenia produced by piribenzamine in normal rats.

In rats with spontaneous goiter piribenzamine did not produce eosinopenia.

Thyroidectomized rats or with spontaneous goiter, when injected during 7 to 9 days with 3-5-3' tri-iodo-tyronine showed a normal eosinopenic reaction to piribenzamine.

Changes observed in the rat testicle due to ligature of vascular pedicle. E. FELS AND G. E. BUR. *(Instituto de Maternidad "A. Peralta Ramos", Buenos Aires).*

The results are presented of the ligature of the testicular vascular pedicle in 23 male rats; 11 with ligatures of the spermatic vasa and 12 with ligature of the whole cord. There was no great difference between these two groups respecting the appearance of hyperplasia of the Leydig cells.

In 16 cases subcapsular micro nodules of true luteoid or tubular hyperplasias were seen very similar to these produced by the ovary, ligated, or grafted into the spleen. The genital atrophy, the absence of a hypophysis of castration and the hyperplasia of the mammary gland in some cases, leads to the supposition of the existence of and endocrine secretion of estrogenic effect by the neoformed tissue in the testis.

This tissue has no faculty to continue growing when grafted which makes us believe that we are not dealing with true tumours.

Thyroid hormones and toad's metabolism. A. O. DONOSO AND J. C. TRIVELLONI. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Paraguay 2155, Buenos Aires).*

1) At 25° C constant temperature and standard conditions, the toad oxygen consumption was of 53.4 cc (40-80) per kg and hour.

2) The thyroid hormones thyroxine and triiodothyronine produced a sensible metabolic augmentation in single dose of 1 mg or in accumulative dose of 0.25 mg during 4 days.

3) The hypophysectomy did not produce modifications until the asthenia period in which diminished, getting more important as far as death.

4) By above data, it is clear that the toad keeps sensibility to the thyroid hormones in high dose, but the fact of the hypophysectomy don't modify the oxygen consumption, makes doubtful the metabolic role of the thyroid in the adult toad.

Hypoglycemic action of phenethyl-diguanide. B. A. HOUSSAY AND J. C. PENHOS. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

Phenethyl-diguanide has a hypoglycemic action in rats, dogs, cats, rabbits, guinea pigs and normal toads.

Adrenalectomized rats were more sensitive than the hypophysectomized and the hypophysectomized more sensitive than the normal animals. Adrenaline, hydrocortisone or glucose had a protective action on the adrenalectomized rats treated with mortal doses of DBI (200 mg/kg).

Rats previously treated with alloxan showed hypoglycemia when they were given DBI (50 mg/kg).

It was observed that in dogs receiving high doses of DBI, the toxic effects of the substance were much more predominant than the hypoglycemic ones.

No changes were observed in the blood sugar level of toads deprived of pancreas or liver when treated with DBI (50 mg/kg).

Radiactive iodine and thyroid function in the toad. A. O. DONOSO AND J. C. TRIVELLONI. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Paraguay 2155, Buenos Aires).*

1) The radiactive iodine uptake in the toad's thyroid is very low and reaches values of only a 9.3 % at 72 hours.

2) It can be augmented still a 56 % by the injection of toad's *pars distalis* and ostensively lowered by the extirpation of that lobe (56 %).

3) Using paper chromatography and autorradiography in animals being injected with I^{131}, mono and diiodothyrosine, iodine and thyroxine, have been detected. The proportion of thyroxine was very small, reaching only a 17 % as a maximum at 96 hours.

4) Thyroid origin substances in plasma, could'nt been detected.

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(2) WHITTEMBURY, G., RAMÍREZ, M., FERNÁNDEZ, J., MONGE, C.: *Acta physiol. lat. amer.*, 1955, *5*, 117.

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metro	m	m	milisegundo	ms	msec
centímetro	cm	cm	litro	l	l
milímetro	mm	mm	centímetro cúbico	cm^3	cc
micrón	μ	μ	mililitro	ml	ml
milimicrón	$m\mu$	$m\mu$	kilogramo	kg	kg
Angström	Å	Å	gramo	g	gm
microgramo	μg	μg	miligramo	mg	mg
gama	γ	γ	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
segundo	s	sec	Microcurie	μC	μC
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